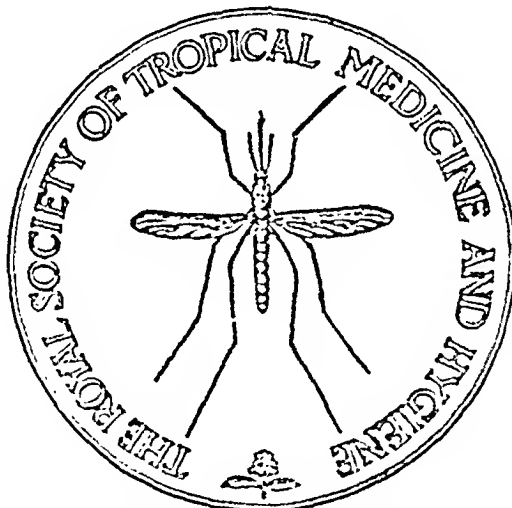# TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

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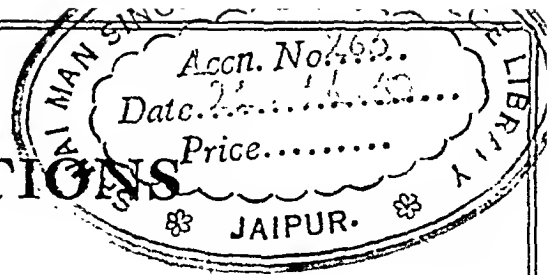ZONAE TORRIDAE TUTAMEN

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OF THE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 16th May, 1940, at 4.30 p.m.

THE PRESIDENT

Sir S. Rickard Christophers, ·C.I.E., M.B., F.R.S., Colonel I.M.S. (retd.)

in the Chair.

PAPER.

A REVIEW OF IMMUNIZATION AGAINST HUMAN RICKETTSIAL DISEASES.

BY

FREDERICK MURGATROYD, M.D., F.R.C.P.

CONTENTS

Just over 30 years ago RICKETTS (1909), later to lose his life during his researches, first described the organisms whose name now immortalizes his memory. Since that time other rickettsias have been discovered and they form an interesting group of organisms responsible for important diseases of man and animals between which they are transmitted by various arthropods.

In man, rickettsial diseases are usually serious and not infrequently fatal illnesses for which no simple and adequate remedy is known but from which recovery is accompanied by the acquisition of considerable immunity to rein-fection, second attacks being rare or mild. It is not surprising, therefore, that many workers have attempted to prepare protective vaccines. In this pursuit a number of peculiar difficulties are encountered and, in order to appreciate

the steps that have been taken to overcome such difficulties, certain preliminary considerations are necessary.

Preliminary Considerations.

Classification of the Diseases.

These infections can be classified, within certain limits, by such features as their epidemiology; their clinical, pathological and immunological effects in man and experimental animals; and their behaviour in appropriate arthropods. In the following classification reference will be confined to characters concerned with the problems of vaccination. The main features in this connection are outlined in Table I.

1. *Typhus group.*—This contains firstly classical epidemic typhus or typhus exanthematicus, a severe disease transmitted from man to man by lice ; and secondly the various varieties of murine typhus, usually more benign infections, normally transmitted sporadically from rats or mice to man by rat fleas. In certain circumstances murine infections may become epidemic and be passed from man to man by lice, and ZINSSER (1937) suggests that in ages past such a process probably led to the development of classical typhus from a murine strain.

The close relationship between the viruses of classical typhus (*Rickettsia prowazeki*) and those of the murine typhuses (*R. mooseri* or *R. prowazeki* var. *mooseri*) is reflected in the Weil-Felix reaction, agglutinins against strains OX 19 and OX 2 of *B. proteus* but not against strain OX K, developing in each type of infection. Considerable, even if incomplete, cross immunity exists therefore between these infections, and is turned to advantage in the preparation of vaccines.

Murine virus in male guineapigs produces fever accompanied by a characteristic reaction in the tunica vaginalis rich in rickettsias (Neill-Mooser reaction). With the virus of classical typhus these animals usually suffer only slight temperature or the infection may remain inapparent, and the scrotal reaction is absent unless the resistance of the animals is artificially lowered as by a vitamin deficient diet. In rats, murine virus produces fever, sometimes scrotal reactions and, after resistance is lowered as by irradiation with X-rays, rich peritoneal accumulations of rickettsias. The classical virus gives rise to only inapparent infections or rarely short fever during which the virus multiplies without causing symptoms and then dies down ; rich accumulations do not occur in irradiated rats.

2. *Spotted fever group.*—This contains Rocky Mountain spotted fever (*R. rickettsi*), fièvre boutonneuse (*R. conori*), and other forms of tick typhus ; the organisms are transmitted to man by ticks from various animal reservoirs.

These infections form a second immunological group and give rise to only inconstant and low-titre agglutinins against the different strains of *B. proteus*.

In guineapigs, they produce scrotal reactions which are very severe and often gangrenous with the Rocky Mountain fever virus but are less constant with the viruses of fièvre boutonneuse and other tick typhuses. Rats usually suffer only inapparent infections.

3. *Tsutsugamushi fever group.*—This contains Japanese river fever or tsutsugamushi fever, the closely related if not identical rural scrub typhus of Malaya, and other forms of mite typhus ; the infection (*R. orientalis*) is transmitted to man by mites from various reservoirs such as field rodents.

(*R. quintana*) being transmitted by lice from man to man. Similar infections such as Weigl's disease (*R. weigli*) and certain recurrent fevers in Russia, Poland, Japan, France and Spain have been observed from time to time since.

This group appears immunologically distinct and MOSING (1936) reported no cross immunity between Weigl's disease and classical typhus.

The susceptibility of laboratory animals is uncertain. BRUCE (1921) in the *Final Report of the War Office Trench Fever Investigation Committee* states that LEDINGHAM observed suggestive fevers in guineapigs and rabbits inoculated with trench fever virus. German workers also claim to have transmitted the infection to mice (JUNGMANN, 1916 ; JUNGMANN and KUCZYNSKI, 1917 ; STRISOWER, 1918 ; not confirmed by TOEPFER, 1916 ; MUNK and

TABL

SUMMARIZING HUMAN RICKETTSIAL INFECTION

Group.	Disease.	Vector.	Organism.	Reservoir.	Weil-Felix Reaction.
HUS ..	EPIDEMIC or CLASSICAL TYPHUS (usually severe). Brill's disease (mild late manifestation) ? Thromboangiitis obliterans (? cryptic chronic infection) GOODMAN (1916, 1937)	LOUSE	R. prowazeki	MAN	OX 19 + + + O 2 + + OX K — ⎫⎬⎭ Cross immunity
	ENDEMIC or MURINE TYPHUS (usually relatively benign. Epidemic-form may occur ; may be severe. Murine typhus—widespread Ship typhus—Toulon Urban shop typhus—Malaya Etc.	RAT FLEAS (Louse in epidemics)	R. mooseri (R. prowazeki var. mooseri)	RATS and mice (? Other rodents, cats or dogs) (Man in epidemics)	OX 19 + + + OX 2 + + OX K — ⎫⎬⎭
TTLD FIVIR..	ROCKY MOUNTAIN SPOTTED FEVER Western type and Sao Paulo fever (severe) Eastern and Minnesota type (benign)	TICKS	R. rickettsi	WILD RODENTS (? dogs and sheep)	OX 19 + OX 2 + OX K + (Variable low titres)
	FIEVRE BOUTONNEUSE — Mediterranean, Kenya Tick Typhus—S. Africa, India, etc.	„ „	R. conori	DOG	
TSUTSUGAMUSHI FEVER	TSUTSUGAMUSHI FEVER (Japanese River fever) Rural scrub typhus—Malaya Mite typhus—Sumatra, Australia, etc.	MITES	R. orientalis	FIELD RODENTS	OX 19 — OX 2 — OX K + + +
NCH FIVIR..	TRENCH FEVER ? Weigl's disease and similar fevers of Russia, Poland, Japan, France and Spain.	LOUSE	R. quintana (? R. weigli)	MAN	
HER RICKITTSIOSES	Q fever—Australia X fever—Montana ? Trachoma (BUSACCA, 1933 and CUENOD, 1936)	TICKS ? Louse	R. burneti ? R. trachomae	? (Bandicoots) ?	

DA ROCHA-LIMA, 1917, and WERNER, 1919), to cats (STRISOWER, 1918) and to guineapigs (MUNK and DA ROCHA-LIMA, 1917); MOSING did not find *R. weigli* pathogenic for these animals. OGATA (1935) infected rabbits intratesticularly with the virus of a trench fever-like disease observed in Japan.

5. *Other rickettsial diseases.*—The position of Q fever of Australia (*R. burneti*), its counterpart, X fever of Montana, and other similar infections is not clearly defined. These infections somewhat resemble murine typhus but no rash is present, the Weil-Felix reaction is consistently negative with the different strains of *B. proteus*, the Neill-Mooser reaction is negative and rats develop only inapparent infections.

The rickettsial origin (*R. trachomae*) of trachoma, suggested by BUSACCA (1933) and

POINT OF VIEW OF VACCINATION.

| ll-Mooser ction in incapigs. | Animal Reactions. | | | Vaccines already used in practice. | | Present developme |
	Experimental infections in rats.	Lung in- fections in white mice.				
	Inapparent infec- tion	Heavy	WEIGL's louse vaccine (killed)			
al	Rich infections in irradiated rats	Heavy	BLANC's biliated guineapig vaccine or Blanc and Baltazard's flea faeces vaccines (attenuated living) NICOLLE and LAIGRET's dried coated guineapig-rat vaccine or Laigret and Durand's mouse - brain passage modification (attenuated living) ZINSSER and CASTENADA's rat vaccine (killed)	Cross protection if antigen sufficient- ly powerful as with living vac- c.nes	Tissue culture, egg ture and lung-infe vaccines being dev ed against vari viruses
racteristic			SPENCER and PARKER's tick vaccine (killed)			
s occur	Rate of inapparent infections	Heavy				
un marren						
jurnt	Usually inapparent infections					

CUÉNOD and NATAF (1936), and its relationship to the rickettsias found by COLES (1931, 1935 and 1936) in conjunctivitis of animals are still matters of controversy.

THE RICKETTSIAS.

The rickettsias infecting man approach each other closely in size, form and staining reactions so that simple morphological differentiation is difficult, although the trench fever and Q fever organisms appear generally stouter and shorter than those of the typhus group.

They are minute, non-motile, non-sporing polymorphic organisms whose dimensions are of the order of 1μ and less ; they appear in short bacillary or diplobacillary forms resembling minute cocco-bacilli or in longer straight or curved filaments approaching in · size the smaller bacteria. They are coloured poorly with most ordinary stains but may be well demonstrated with Giemsa's strain, or by appropriate techniques such as those of CASTENADA or MACCHIAVELLO (1937).* The organisms are frequently found in masses, and bipolar and diploid forms may represent stages in division. They do not grow in ordinary media but seem to require some form of tissue culture ; in the vertebrate host their development is for the most part intracellular, and in an appropriate arthropod their multiplication may be relatively enormous.

Reference has already been made to the rich infections which are produced by murine strains of typhus in the tunica vaginalis of guineapigs, and which can also be obtained in the peritoneum of rats whose resistance has been artificially lowered ; such infections are useful as sources of virus in the preparation of certain vaccines. Another method of obtaining high concentrations of virus is to utilize the characteristic development that occurs in suitable arthropods, and as this is also employed for the preparation of vaccines these arthropod infections will be outlined briefly.

ARTHROPOD INFECTIONS.

The various rickettsial infections are normally transmitted by distinctive vectors but this predilection is not absolute. For example, the normal invertebrate host of *R. prowazeki* is the human louse *Pediculus humanus*, but the lice of the monkey and the lice of susceptible animals such as guineapigs or rats may transmit the virus between the respective animals (BURNET, 1937). Transmission of murine virus by the louse has been demonstrated by MOOSER and DUMMER (1930), LÉPINE and BILFINGER (1934), SPARROW (1939) and others, while classical typhus can also be transmitted by the flea. The virus of Rocky Mountain spotted fever is normally transmitted by the ticks *Dermacentor andersoni* and *D. variabilis*, but other acarines can serve as vectors, and WEIGL (1930a) has shown that the virus can develop in the louse. Similarly the virus of Japanese River fever can be transmitted experimentally by lice or fleas, although the normal vector is the mite *Trombicula akamushi*. These

* Stain smears for 3 to 5 minutes with 0·5 per cent. aqueous basic fuchsin (pH 7·2 to 7·5), rinse rapidly with 0·5 per cent. citric acid, wash and counterstain for 1 or 2 seconds with 1·0 per cent. aqueous methylene blue,

experimental variations do not produce fundamental changes in the viruses and ZINSSER and MOOSER, according to BURNET (1937), have never, despite many years of effort, produced a permanent modification even between murine and classical virus by such means.

Lice.—Human lice infected by feeding on patients suffering from classical typhus begin to pass *R. prowazeki* in their faeces about 5 to 9 days after the infecting feed. It is by such faeces entering skin abrasions such as those caused by scratching or by the louse in feeding, by the faeces reaching some mucous membrane such as the conjunctiva, or perhaps by their being inhaled or ingested, that man becomes infected ; crushing lice by scratching may, it is said, also liberate virus and lead to infection. During development in the louse, the rickettsias invade the epithelial cells of the louse's gut where they multiply and distend the cells until these are destroyed thus liberating masses of organisms, so that after about 10 days the lumen of the gut becomes filled with almost a pure culture of rickettsia. From 10 to 100 million organisms are produced in each louse and in about 12 days practically all the cells are affected and the louse dies. The organisms do not invade the body cavity or the salivary glands of the louse, and the infection is not transmitted hereditarily. Experimental infections of lice with the virus of murine typhus show no gross differences from those with classical virus ; the more rapid evolution of infections with murine virus and the earlier death of the lice are explicable in terms of different experimental procedures according to LEVADITI and LÉPINE (1938). STARZYK (1938) found *R. prowazaki* remained virulent in lice or their faeces dried for several months. PCHENICHNOF and RAIKHER (1936) state that lice may infect each other by buccal contamination with *P. prowazeki*, and they suggest that by this means also the virus might be maintained in interepidemic periods without the intervention of human disease ; presumably the human hosts of such lice must be immune to infection.

The organism of trench fever (*R. quintana*) undergoes development in the lumen of the louse's intestine forming a layer on the gut cells which are not invaded, and this is also the case with the organisms described by MOSING (*R. weigli*) and HERZIG (1939) in more recent outbreaks resembling trench fever. A similar extracellular organism (*R. pediculi*), described in otherwise normal lice, is said by MOSING to be non-pathogenic to man and antigenically distinct from *R. weigli*, but further observations are required to determine the precise relationships of these various extracellular organisms.

The rickettsia of Rocky Mountain spotted fever (*R. rickettsi*) when experimentally introduced into the louse can also multiply as shown by WEIGL, but the infection remains of the *R. quintana* type, the intestinal cells not being invaded and the louse showing no morbid symptoms.

There is a rickettsia (*R. da rocha-limae*) which causes epidemics among lice but which does not give rise to disease in man. During development it occupies partly an intracellular and partly an extracellular position in the louse gut, and is readily transmissible to clean lice by contact with infected lice.

Fleas.—In fleas infected with the virus of murine typhus (*R. mooseri*) the organisms are found in abundance in the lumen of the intestine ; hereditary transmission does not occur. BLANC and BALTAZARD (1937) have shown that infected fleas remain infective even if starved for long periods, and ZINSSER states that fleas may harbour virus for over a month and remain alive.

PRINCIPLES OF VACCINATION.

In some respects, the rickettsias appear to stand between the ordinary bacteria and the ultra-viruses so that it is not altogether surprising to find that, when killed, they lose to a very considerable extent their power of stimulating immunity. It appears necessary, therefore, for successful vaccination, to use :—

1. Living organisms of normal virulence in small or graduated doses,

2. Living organisms whose virulence has been attenuated in some way, or,

3. Killed organisms in very large numbers in order to stimulate a sufficient antibody response.

TABLE II.

OUTLINING RICKETTSIAL VACCINES.
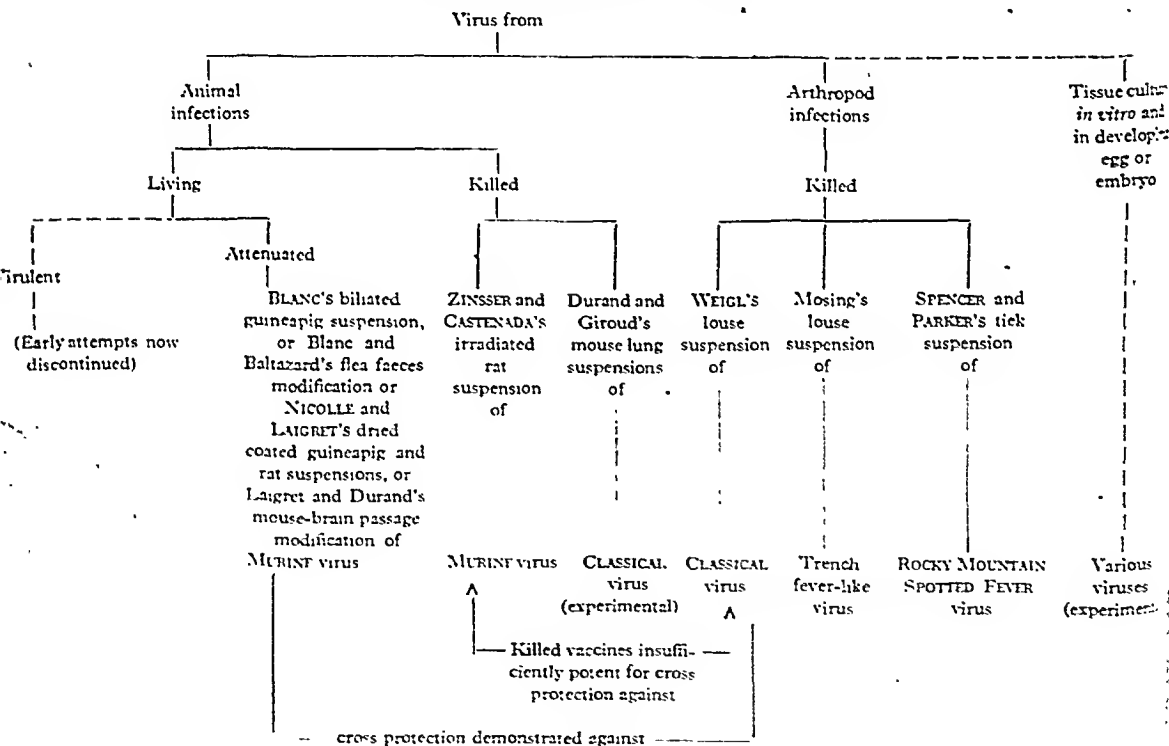
The first method is accompanied by obvious risks and is no longer used in practice.

The second may also not be free from danger when a very virulent virus is being used, but taking advantage of the cross immunity that exists between the relatively benign murine typhus and the more serious classical typhus,

vaccines of living but attenuated murine organisms have been successfully employed in practice to protect against either type of infection.

The third procedure of using killed organisms, attractive by reason of its safety, is impeded by the failure of rickettsias to grow in ordinary culture media and the consequent difficulty of obtaining concentrations of organisms sufficiently large to possess any practical immunizing power when killed. This obstacle has been circumvented by utilizing the comparatively rich suspensions that may be obtained after the organisms have undergone multiplication :—

1. In the appropriate arthropod host,
2. In some suitable laboratory animal, or
3. In tissue culture.

Utilizing one or other of the principles set forth above, various vaccines have been prepared and, although none is free from some criticism, considerable success has been obtained in practice. Table II (page 8) gives an outline of the vaccines used which will now be described in more detail.

It may be here remarked, in view of the infectivity of rickettsial diseases and the many serious and sometimes tragic accidents that have befallen workers with these fevers, that investigations should only be carried out under proper conditions with all due precautions, and that the whole of the personnel engaged should be immunized as adequately as possible against the strains being handled, a suggestion supported by NICOLLE and SPARROW (1932).

therefore attempted vaccination of man by these routes with living murine virus, but such methods do not commend themselves as frank infections occurred. Nevertheless the intranasal instillation of virus into a suitable experimental animal as an intermediate step in the preparation of a vaccine has recently led to work of considerable interest.

Living vaccines may be not only dangerous to the individual but also to the community. ILCHUN YU (1931) showed that patients convalescent from typhus might be capable of infecting lice, and KUTEISCHIKOW, DOOSER and BERNHOFF (1933) found that the blood of patients who had previously had typhus might become virulent after the patients had been bitten by infected lice. Thus typhus virus may circulate in an individual for a time despite some degree of immunity, and modern views on Brill's disease suggest that once the living virus is introduced into man it may remain alive for a considerable time.

It may be said, therefore, that the dangers and difficulties associated with virulent living vaccines preclude them from use in practice.

Attenuated Vaccines.

Blanc's biliated vaccine.—The first important advance in the use of attenuated living vaccines followed the work of BLANC and his collaborators (1933 and 1934). They concluded that it would be possible to vaccinate successfully with a living vaccine, provided some practical method could be devised for obtaining a vaccine neither dangerous nor capable of giving rise to carriers of virus who might infect lice. In the end they used a strain of murine virus isolated in Casablanca and, following work on the attenuation of dengue virus, treated the virus with ox bile. The term attenuation, according to BLANC (1937), only implies that the virus has become innocuous when used as a vaccine, due to some physico-chemical change, perhaps a coating of bile, retarding absorption; he does not believe that biliation either partially destroys or permanently modifies the virus.

The tunica vaginalis, spleen and suprarenal of guineapigs infected with murine typhus are emulsified in physiological saline about the 2nd or 3rd day of the disease, a final tissue concentration of about 1 : 2,000 being obtained (previously 1 : 1,000 was used). To this suspension, after filtration through gauze, is then added sterilized ox bile to the extent of five parts bile per 100 emulsion and the mixture is allowed to stand for 15 minutes. The biliated mixture is then inoculated intramuscularly without further delay, the dose now employed being 1 c.c. per person. About 2 litres of vaccine is prepared from each guineapig.

BLANC and his colleagues (1935) believe that biliated vaccines, before immunizing, produce a state of premunition and that after about the 12th day a second dose of virus, pure or attenuated, gives rise to the development of comparatively solid immunity. For the control of epidemics a single dose producing premunition probably suffices, but between epidemics a second injection is desirable to enforce a prolonged immunity.

In an early series of experiments nineteen persons were tested by inoculation with murine typhus 25 days after vaccination and none became ill; six of them

were then further inoculated with classical typhus without untoward result. In view of this, six other vaccinated persons were tested directly with classical virus, heavy doses being given 1 or 2 months after vaccination, and again the results appeared entirely satisfactory (BLANC, 1937).

BONJEAN and NAIN (1937) found positive Weil-Felix reactions, the titres varying from 1 : 25 to 1 : 500 with the majority at 1 : 25, in ninety-six out of 111 people vaccinated 14 to 25 days previous to the examination ; the remaining fifteen reactions were negative. Control examinations before vaccination were not apparently made.

Owing to the cross immunity between murine and classical typhus, the vaccine leads to the rapid arrest of epidemics of typhus exanthematicus in a vaccinated population. BLANC and GAUD (1935) reported that vaccination was carried out in two villages, one in conjunction with delousing and one without delousing, but that in each case the outbreaks came to an abrupt end. Again, in Petitjean after vaccination the typhus epidemic stopped, although no prophylactic measures other than vaccination were taken. Blanc's vaccine has now been employed on a large scale, and GAUD (1938) states that more than a million people have been vaccinated in Morocco where the vaccine has given excellent results and brought about a complete arrest of typhus in epidemic foci. In the opinion of LEVADITI and LÉPINE (1938) the only criticism that can be directed at the biliated vaccine resides in the possibility of its producing febrile vaccinial reactions, but in Africa these appear to have been few and benign. In Chile, however, the results appear to have been less satisfactory where, among 800 vaccinated, 23 per cent. showed a picture of grave typhus and five died ; virus was isolated by animal inoculation from the blood of patients with vaccinial typhus (PALACIOS, CHAVEZ and AVENDAÑO, 1935). This may have been due to a greater sensibility of the Latin American population to murine virus or possibly to some deviation in technique.

BLANC states, however, that vaccination does not create foci of murine typhus because fleas or lice are incapable of being infected by the vaccinated whose infections remain constantly inapparent and benign. Whether or not this is true in those suffering reactions may be another matter, and although the risk of inducing benign murine infections may perhaps be disregarded in the face of epidemics in areas where typhus already exists, the use of the vaccine for prophylaxis in areas free from typhus would require careful consideration.

The technique of producing the biliated vaccine must be accurately followed and a further disadvantage is that it must be carried out just before use. LAURENS, FORT and BERNIER (1939) had certain failures in military practice which they attributed to the impossibility of keeping the virulence of the vaccine constant and to its rapid loss of efficacy. They suggest that these difficulties might be overcome by using the dried excreta of infected fleas as a source of virus, according to the method of BLANC and BALTAZARD (1938, 1939 and 1940), who collected and dried the faeces of fleas fed on infected rats.

Blanc and Baltazard's modification.—These authors prepared a biliated vaccine by the following method.

The fleas (*Xenopsylla cheopis*) are contained in special bins, 50,000 in each, and are infected over a period of 2 weeks by continuous contact with a series of rats infected with murine typhus ; the rats in pairs are infected 48 hours before being placed in the bins where they remain 2 days, being then replaced by another pair. At the end of a fortnight all the fleas are infected and the hair of the rats becomes heavily contaminated with the infected faeces of the fleas. As the rats die or are sacrificed at the end of their 2 days' sojourn in the bins their hair is removed and dried *in vacuo* over calcium chloride, after which the faeces are easily separated by sieving. The dried faeces are then sealed in ampoules *in vacuo* and in this condition retain their virulence for more than a year and a half if stored in the refrigerator. In practice the faeces of several yields are pooled and using a number of bins 40 grammes of dried material could be obtained each month without difficulty. For use it is dissolved in buffered saline containing 1 : 150 sterilised ox bile, so that the individual dose of 1 c.c. represents 1/100 mg. dried faeces.

Reports are available of some 271,666 vaccinations with only eight vaccinial reactions ; thirty Europeans vaccinated suffered no local or general reaction even with triple doses. Tests after vaccination suggest the method is efficacious.

Nicolle and Laigret's dried coated vaccine.—These authors (NICOLLE and LAIGRET, 1935, 1936), employing a technique used in the preparation of a yellow fever vaccine, attenuate the virus by drying, and retard its absorption by coating with egg yolk and oil.

Guineapigs or rats infected with a Tunisian strain of murine virus are sacrificed on the 2nd day of their fever. The brain, after standing 24 hours in neutral glycerol in the refrigerator, is triturated with 2·5 grammes of a mixture consisting of 100 parts anhydrous disodium phosphate and 15 parts potassium dihydrogen phosphate. The triturated mass is then desiccated at 5° C. *in vacuo* over calcium chloride for 24 hours (sulphuric acid or phosphorus pentoxide must not be used as they kill the virus). To the dried powder is then added drop by drop 8 c.c. of sterile egg yolk, previously submitted to a temperature of 56° C. for an hour on 3 consecutive days. This mixture is triturated, desiccated during 24 hours and the resulting powder sealed *in vacuo* in ampoules, each dose containing 0·05 gramme which represents one 200th part of the brain ; compressed tablets, equivalent to twenty doses, are also prepared. The vaccine is preserved at −15° C. and will keep potent for a month although in practice it is renewed fortnightly ; at ordinary temperature it may only be relied upon for 48 hours. Tests for virulence and bacterial sterility are imposed at each stage of the procedure. Each dose, immediately before use, is suspended by rubbing up in 1 c.c. of olive oil (neutral, washed in alcohol and sterilized), added drop by drop. A dose equivalent to 1 : 200 guineapig brain is given, followed by another equivalent to 1 : 200 rat brain at an interval of 25 to 30 days ; in the routine control of typhus the first dose may be omitted. The inoculum is often mixed with T.A.B. vaccine.

LAIGRET, DURAND, BELFORT and LEFAUCHEUR (1937) give a critical examination of the results of vaccinating 32,481 persons against typhus exanthematicus by means of this murine vaccine. There were no serious accidents, and the great majority of persons developed only inapparent infection which is the essential mechanism of the vaccination. The Weil-Felix reaction remained negative in most cases, but about 3 per cent. showed agglutinins against *B. proteus* (titres of 1 : 50 to 1 : 400) without having had any fever ; the reaction was usually positive in those who had fever even when that fever was due to some other.

cause than typhus. Five cases showed symptoms of murine typhus 10 to 16 days after vaccination but vaccinial typhus was confined to town-dwellers, and above all to Europeans, no case occurring among the Arabs of the country. The authors remark that this is of interest from the military point of view ; they suggest that all indigenous troops should be vaccinated and, in the case of the European personnel exposed to risk, vaccination should also be undertaken without hesitation, provided warning is given of the possibility of febrile vaccinial typhus, rare though this may be.

In all the epidemic foci, the epidemics came to an end 3 weeks after vaccination and the best results were obtained when vaccination was combined with delousing. In one epidemic, out of ninety-one typhus contacts, attacks occurred in 100 per cent. who were neither deloused nor vaccinated ; in 72 per cent. who were deloused but not vaccinated ; and only in 8 per cent. who were both deloused and vaccinated, and these 8 per cent. were already in the incubation period when vaccinated. If vaccination alone is employed during an epidemic some cases of typhus must be expected until the end of the third week.

The duration of immunity conferred by the vaccine is not known with certainty, but six groups inoculated in 1935 to 1936 remained free from infection during the epidemic of 1937, although neighbouring non-vaccinated populations suffered severely. In Tunis, three cases of typhus are known in persons previously vaccinated and, more than a year after vaccination, a small number of mild cases in Algeria. It is not uncommon to see in epidemic foci certain of those vaccinated presenting short mild fevers, followed by positive Weil-Felix reactions, entirely comparable to the fevers of reinfection that may occur in those who have previously suffered from a natural typhus infection. However, authentic cases of typhus in those vaccinated have so far not only been very rare but have never been fatal. Furthermore, where vaccination is practised, isolation and quarantine may be confined to those actually suffering from typhus, markets may remain open and the economic life of the population spared serious dislocation.

As with Blanc's vaccine, the disadvantages of this vaccine are the complication of its preparation, its poor keeping properties and the possibility of its producing occasionally frank infection. In endemic areas or in the face of epidemics these shortcomings seem slight in view of the satisfactory protection apparently given. LAIGRET and DURAND (1939) report that over 100,000 vaccinations have been carried out and that areas previously the site of recurrent epidemics have remained silent for more than 3 years.

Laigret and Durand's modification.—These workers (1939) have improved the original method, however, by utilizing a mouse-brain passaged murine virus which can be more accurately titrated and which is more stable to physical and chemical agents. The virus only requires a single coating (egg yolk) and can be preserved for some months in the refrigerator and for at least a week at ordinary temperatures. For use it is suspended in water and a single dose of ten mouse-units is given to the indigenous country people, but for more

susceptible urban or European populations three graduated doses are used, the first being killed by heat, the second after a week corresponding to one mouse-unit and the third after another 3 weeks being equivalent to ten mouse-units. Each mouse provides about 1,000 doses and over 7,000 vaccinations were carried out successfully from the point of view of innocuousness and efficacy.   More recently LAIGRET (1940) gives further details of the use of this vaccine administered subcutaneously and also by cuti-puncture intradermally.

Killed Vaccines.

Numerous early workers attempted to employ blood or emulsions of organs from infected patients or animals, as well as suspensions of *B. proteus*, after the infected material had been treated by heat or antiseptics but these experiments only seemed to confirm the difficulty of producing any lasting immunity with killed vaccines.

DA ROCHA LIMA (1918) however found that repeated injections into guineapigs of phenolized emulsions of lice infected with classical typhus conferred a certain amount of immunity, although this was not confirmed by DOERR and SCHNABEL (1919).   BREINL (1924) using the intestinal contents of infected lice, obtained results promising in rabbits but not so good in guineapigs ; and MARTINI (1919) seemed favourably impressed by a louse-vaccine combined with immune serum.   It may here be observed that DYER, WORKMAN, RUMREICH and BADGER (1932) also experimented with phenolized vaccines prepared from fleas infected with murine typhus.

Weigl's louse vaccine.—The really important practical application of DA ROCHA LIMA'S work was however due to WEIGL (1930b), who developed the following method.

Laboratory reared lice, artificially infected with classical virus per anum by means of a fine capillary pipette under the microscope, are fed twice daily on immune persons until, in about 10 days, the rickettsias have multiplied so that each louse contains from 10 to 100 million organisms. The infected intestines, normally sterile excepting for the rickettsias, are dissected out and emulsified in saline containing 0·5 per cent. phenol. Three standardized suspensions were made containing 1,250 million, 2,500 million and 5,000 million organisms per c.c. respectively (equivalent to about 25, 50 and 100 louse intestines), and 1 c.c. of the respective dilutions were given subcutaneously in series at 3 to 6 day intervals.

It is obvious that there are very considerable technical difficulties in the preparation of this vaccine and, despite the model organization and the high skill of the personnel existing until recently at Lwow, it was not possible to produce more than 2,000 doses per month, representing the use of several thousand lice daily.

In an attempt to reduce cost and increase production, the number of intestines has been lowered successively from 175 to 90 for each set of vaccine without unduly diminishing its practical efficacy. As the rickettsial content of the intestine may vary from 10 to 100 million, intestines coming from a large stock of lice, over a period of 3 to 5 months, are pooled thus giving a fairly constant concentration of 5,000 to 6,000 million rickettsias for a standard vaccine (three injections) of ninety intestines.

In the control of epidemics, even smaller doses may be used and for this purpose a

vaccine of only ten intestines was in successful use, the higher titre vaccines being reserved for medical personnel and those exposed to high risks. The vaccines may be preserved in the refrigerator for 1 to 2 years.

There is usually only slight local redness and tenderness with rarely any general reaction following vaccination, and protection is said to be given for 2 to 3 years.

LIU, ZIA and WANG (1938) studied the serological response of individuals after vaccination and found that, while both Weil-Felix reactions and typhus rickettsial agglutinins were obtained with the majority of sera from subjects recently vaccinated, no parallelism or constancy between the titres of the respective antibodies was observed. The *Rickettsia* agglutinins tended to wane more rapidly than the *Proteus* agglutinins.

WEIGL states that the protection conferred on laboratory workers is remarkable, those being vaccinated remaining unharmed despite the bites of hundreds of infected lice. NICOLLE and SPARROW (1932) failed to infect two vaccinated children with virulent emulsions of guineapig brain. CHODZKO (1933) writes that in Poland only a single case, and that doubtful, occurred among 2,794 persons vaccinated in 1931-2 despite the fact that they were particularly exposed to risk of infection being physicians, nurses or family contacts of typhus patients. TCHANG and LOTSONG (1934) describe a Belgian mission on the border of Mongolia and China where four or five out of 180 missionaries were lost each year from typhus, the disease being responsible for two-thirds of the total mortality. After vaccination, not a single death occurred among those vaccinated during the period 1931-6 (RUTTEN, 1936). RADLO (1937) reports that in the Jawarow district of Poland where typhus is endemic and breaks out in serious yearly epidemics, 13,980 persons were vaccinated from 1933-6, a quarter receiving doses equivalent to 1-10 intestines, half 10-20, and the remaining quarter 10-90; no other anti-typhus measures were employed. Before vaccination there was a yearly average of 200 cases, but during the 4 years under review a total of only sixty, of which fifty-five occurred during or immediately after vaccination. Of the remaining five cases, one had been vaccinated with only one intestine 8 months previously, two with 4-5 intestines 2 months and 3 years previously, and two with 90 intestines 2 months and 1 year previously respectively; all the attacks in the vaccinated were benign. It was found that vaccination of only 30 per cent. of the population with small doses (1 to 10 or 20 intestines) sufficed to arrest the development of the epidemic at the end of 4 weeks. DRBOHLAV (1938) states that in infected areas of Slovakia and Sub-Carpathia Weigl's vaccine was used during 1934-7 and that afterwards only sporadic cases were found. MARIANI (1939) reports that among 13,000 persons vaccinated in Ethiopia, only a few cases of typhus occurred and these were mild with one exception in which death occurred, but in this case, as in four of the others, infection was acquired before vaccination started

The most serious obstacle to the use of Weigl's vaccine is the labour of its production, CHRZANOWSKI and MOSING (1933) used phenolized suspensions of the excreta of infected lice as vaccines, but although this may obviate the dissection of the lice or may add to the yield of organisms it does not do away with the difficulties of infecting and rearing the lice.

Zinsser and Castenada's rat vaccine.—In view of the trouble of preparing Weigl's vaccine various workers sought other methods.   TZEKHNOWITZER and PALANT (1933) tried formolized suspensions of infected guineapig brain experimentally and concluded that formolized emulsions gave better results than phenolized vaccines ; but KLIGLER, OLITZKI and ASCHNER (1932) had stated that neither were of much value, that suspensions in distilled water were better, and that the efficacy of the vaccine depended on the presence of a small amount of living virus.   The fact that SPENCER and PARKER (1925) had prepared a satis-' factory phenol-formolized vaccine against Rocky Mountain spotted fever from infected ticks, and the results of Weigl's phenolized louse vaccine showed con- clusively, however, that killed rickettsias were capable of stimulating immunity. ZINSSER and BATCHELDER (1930) also found that guineapigs could be immunized with formolized suspensions derived from the heavy infections which occur in the tunica vaginalis of male guineapigs infected with murine typhus.   In these animals, murine virus, especially at the time of its isolation, produces swelling in the region of the scrotum accompanying the fever.   At the acute stage, there is considerable oedema of the subcutaneous cellular tissue and of the tunica vaginalis with exudation of fluid ; this exudate and the superficial scrapings of the tunica contain an abundance of endothelial cells stuffed with rickettsias (" Mooser cells ").   With the classical virus of epidemic typhus the reaction is rare, although it may occur if the animal's resistance has been artificially lowered.   ZINSSER and CASTENADA (1931 and 1932) found additional peritoneal accumulations of rickettsias could be obtained from rats infected with murine typhus when the resistance of the animals had been lowered by scorbutic diets, by injections of benzol or by irradiation with X-rays.   They, therefore, devised the following method of preparing a vaccine.

Rats were exposed to X-rays for 1 hour under the following conditions : 170 KV. constant potential ; 80 cm. distance ; 0·5 mm. copper filter plus 4 mm. celluloid ; current 8 milliampères ; effective wave length 0·160 Å ; intensity 10 " r " units per minute. They were then immediately inoculated with a suspension of a tunica infected with a Mexican murine virus. The inoculated irradiated rats may die on the 3rd day, but this is too soon for a sufficient accumulation of the rickettsias in the peritoneal cavity from ruptured infected cells, and animals surviving to the 4th or 5th day are used (control irradiated rats sicken on the 4th or 5th day but may live for 8 days). The peritoneal exudate of the infected animals is collected and the peritoneum washed with and gently scraped into 0·2 per cent. formalin in saline ; organisms may also be obtained from the tunica. The suspension is centrifuged, the deposit washed and the pooled cell-free suspensions standardized to contain about 1,000 million organisms per c.c. Three or four doses, ascending from 0·5 to 4 c.c., are given at weekly intervals.

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of three normal, men developed typhus when subsequently inoculated with virus : they admitted that the numbers were small and the previous histories of native Mexicans uncertain, but they were encouraged by the fact that a positive Weil-Felix reaction developed in these and others vaccinated by CASCO. More recently, ZINSSER (1937) states that with such vaccines large scale human immunization has been carried out in Mexico. VEINTEMILLAS (1939) also proposed to use the vaccine on a large scale in Bolivia, having obtained satisfactory results in preliminary experiments. He tested twelve vaccinated people each with a dose of virus estimated at 2,000 minimum infective doses for man, and only three showed a slight fever. He also found guineapigs could be immunized against classical virus with the vaccine but that three to four doses were necessary, whereas for protection against the murine virus a single dose of vaccine sufficed. ZINSSER does not however consider that the murine method completely solves the European problem because of its incomplete protective potency against this infection. As he was unable, with the classical virus, to produce large yields of rickettsias in X-rayed rats, he and his colleagues have now turned to other methods of producing vaccines against this infection which will be mentioned later.

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It seems clear that with a disease like typhus virulent living virus, alone or combined with immune serum, is too dangerous and troublesome for use in routine vaccination.

Killed virus, although obviously safe, has such a limited and enfeebled power of stimulating immunity that large amounts are required, and unfortunately all the methods hitherto used for obtaining adequate concentrations of virus, particularly of classical virus, possess serious shortcomings in practice. The yields obtained by Weigl's technique are offset by the labour involved ; further more, LEVADITI and LÉPINE (1938) state that the duration of immunity conferred by the vaccine is shorter than first suggested and that, even if immunity appears well established for a year, it is uncertain later. No satisfactory practical method has until just recently been described for obtaining adequate concentrations of classical virus from animals and, although good yields of murine virus may readily be obtained from irradiated rats, it has been seen that ZINSSER admits that killed vaccines prepared from such strains give only incomplete protection against epidemic typhus. BLANC (1937) having stated that he and BRUNEAU failed to protect a number of individuals with a phenolized murine vaccine prepared from infected guineapigs, points out that although there is reciprocal

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B

Castenada's murine vaccine to protect against classical typhus has its counterpart in the failure of Weigl's vaccine to protect workers at the Pasteur Institute of Tunis where, out of six cases of murine typhus, four occurred in those vaccinated with Weigl's vaccine. BLANC believes that in view of the negligible action of the killed vaccines on the closely related infections they can only have a feeble action against their own diseases. He concludes that to confer any protection, even feeble and of short duration, with a killed vaccine it is necessary to inoculate enormous doses. NOURY (1937) came to the conclusion, from the results of daily repeated vaccination of guineapigs with phenolized suspensions of infected guineapig brain, that killed vaccines do not protect and that living vaccines are necessary for immunization.

Turning then to vaccines of living but attenuated virus, another set of difficulties is encountered. There are the obvious disadvantages that the vaccines cannot be preserved for any lengthy period and that the process of attenuation requires precise control in order that the virulence may be inhibited without destroying the immunizing power. ZINSSER (1937) goes so far as to suggest that with typhus virus in the form of virulent blood or tissues there exists at present no reliable method for controlled attenuation ; either such material is virulent and consequently dangerous, or else it is killed and has no immunization value. Nevertheless, the French workers appear satisfied that with their attenuated murine strains the immunizing power is adequate and that there is no undue risk in their use. It may not be without significance, however, that these vaccines have for the most part been employed in areas where typhus is endemic, among populations who have been long exposed to risk of infection and who may, therefore, possess some slight inherent or early acquired immunity. REITLER, BTESH and MARBERG (1939) found in Palestine that endemic typhus is a disease of non-immune immigrants and they suggest that the indigenous population is more or less immune through " silent " infection in childhood. LAIGRET and DURAND also recognize this in Tunis with their mouse-brain vaccine, giving a single dose of ten mouse units to the indigenous country folk but three graduated doses, starting even with killed virus, to the urban or European population. It has already been noted that in Chile among the Latin Americans severe reactions were encountered with Blanc's vaccine, and that NICOLLE and LAIGRET'S analysis of their vaccine in North Africa records that cases of vaccinial typhus were observed exclusively following urban vaccinations and above all among the Europeans ; there was none among the Arabs of the country. Although in the presence of typhus it may be preferable to run the risk of a benign reaction or even of a frank attack of murine typhus rather than the risk of the severer classical disease, the use of attenuated living vaccines among populations who have never been exposed to typhus in countries free from infection would call for careful consideration. Particularly would this be so if, as might well be the case with troops in war, such a population was at the same time lousy, for it has been shown that living virus once introduced may persist

in an individual for a considerable period. Lice may become infected by feeding on monkeys suffering from murine typhus and LÉPINE and BILFINGER (1934) found that if the resistance of the animals be lowered by blocking the reticulo-endothelial system and by exposing them to cold during the period of incubation, the proportion of lice becoming infected may rise to 100 per cent. The conditions of these experiments may have been severe and artificial but it is not inconceivable that they may be imitated by a population suffering hardships, injury or various infections as may occur in time of war or other stress. Infections of lice have been observed under natural conditions on patients in Mexico, although it may be argued that the virus here concerned is more akin to classical virus than other murine strains. However, with a Tunisian strain of murine typhus, NICOLLE and LAIGRET (1935) have infected lice on a patient suffering from a febrile reaction following vaccination with living virus and they say that consequently it is prudent to delouse patients before vaccination. Murine infections in lice develop exactly as do those of classical typhus, and although the virus preserves its fundamental characters, producing scrotal reactions in guineapigs and typical fever in rats, there seems a possibility that in certain circumstances living although attenuated vaccines might give rise to vaccinial infections with some danger of their spread. The League of Nations Consultation of Experts (1937) reviewing the position of typhus vaccination for the Spanish Government concluded that living virus vaccines, when they induced infection, conferred greater and wider protection (*i.e.*, protection applying to a greater number of strains or species) than did the same viruses when killed, but recommended that although the risks involved in using living vaccines might be disregarded in the presence of an epidemic, killed vaccines were desirable, in spite of their greater cost or lesser efficacy in a country free from typhus.

SPOTTED FEVER.

Spencer and Parker's Tick Vaccine.—It has already been mentioned that the first successful practical demonstration of protection against a rickettsial infection by means of a killed vaccine was that by SPENCER and PARKER (1935) who used a phenol-formolized emulsion of ground-up infected ticks (*Dermacentor andersoni*) against Rocky Mountain spotted fever.

Larval ticks hatched from the eggs of engorged females are infected by feeding on rabbits infected with spotted fever virus. The infected ticks are reared to adult stage and then fed for 4 or 5 days on guineapigs. These partly engorged ticks are ground up with fine sand in a small quantity of saline containing 1·6 per cent. phenol and 0·4 per cent. formalin. After grinding, the mass is removed to a stock bottle and further phenol-formol saline added until the concentration is about four ticks to the c.c. After standing for 48 hours during which most of the tick protein is precipitated, an equal volume of physiological saline is added and the mixture then kept at room temperature for 7 days. This is then diluted with an equal volume of physiological saline and, by centrifugation, a clear solution obtained which after suitable potency and sterility tests, is used as the vaccine. Two doses, each of 2 c.c., are given subcutaneously at 5-day intervals and are repeated each year before the beginning of the tick season.

The inoculation produces as a rule only slight local symptoms although occasionally there may be headache, some fever and general malaise for 24 to 48 hours ; rarely there may be an urticarial rash but such cases clear up without serious consequences. Protection varies greatly and, for this reason, yearly revaccination is recommended.

PARKER (1935) reported that only sixty-four out of 150,000 vaccinated in the endemic area developed spotted fever. Protection was complete against the mild Idaho virus, the morbidity falling among the highly exposed shepherds from 6 per cent. in the unvaccinated to 0·5 per cent. in the vaccinated, while against the highly virulent Bitter Root Valley virus of Montana, protection was shown by a fall in fatality from 82 per cent. among the unvaccinated to 6·6 per cent. among the vaccinated. Similar protection was given to laboratory workers handling the Bitter Root virus ; infection occurred in twenty-two cases, and of seven not vaccinated all were fatal, whereas among fifteen vaccinated only one died.

MONTEIRO (1933) showed that with the South American tick *Amblyomma cajennense* a similar vaccine could be prepared, effective in guineapigs, against North American spotted fever and Sao Paulo tick typhus, and CANET (1934) also produced a spotted fever vaccine using the European tick *Dermacentor reticulatus*.

OTHER INFECTIONS.

Tsutsugamushi Fever.

Satisfactory vaccines against mite typhus are yet wanting.

KAWAMURA and his colleagues (1937 and 1939) showed that inoculation with a weakly virulent strain (Pescadores strain) of tsutsugamushi fever produced a mild illness in man followed by immunity to inoculation with a strongly virulent strain (Niigata strain), but such a method of immunization is obviously unsatisfactory for use as a routine prophylactic measure.

ANIGSTEIN (1933) in Malaya, cultivated from the blood of patients suffering from scrub typhus or from the tissues of infected animals on blood-agar, rickettsia-like organisms which he then suspended in saline containing 0·3 per cent. formalin. Using 200 and 400 million organisms at a week's interval, he vaccinated 300 coolies, on a highly infected oil-palm estate, and another seventy with only the first dose. During the first half of the year before vaccination there were forty-four cases, but in the second half of the year after vaccination there were only fifteen. Two cases occurred during the month of vaccination, but of the thirteen remaining, nine were among the unvaccinated, three among the vaccinated and one where it was uncertain whether vaccination had been done or not.

LEWTHWAITE (1939) was unable to confer any real immunity against tsutsugamushi fever to guineapigs by means of various vaccines prepared from

suspensions of *R. orientalis* treated with formol, phenol, egg yolk and olive oil, cysteine or combinations of cysteine and formol or phenol.

Trench Fever.

In view of the present war, it may be of importance to mention certain observations on trench fever and allied recurrent rickettsial diseases. During the 1914-18 War trench fever was according to BYAM (1919) responsible for a greater amount of sickness than any other infection on the Western Front, and the *Official History of the Great War* (1921) gives an estimate based upon the year 1917 that a force of a million would lose yearly by evacuation for trench fever some 45,000 men. Of these 80 per cent. would require 2 months at depots or bases and be off duty for 3 months, and of the remaining 9,000 about 2,000 would be incapacitated for more than 6 months. After the War trench fever unexpectedly disappeared before the question of vaccination had received any serious study, although BYAM states that an attempt had been made to vaccinate by means of an emulsion of louse excreta sterilized by heat or phenol.

MOSING (1936) described an interesting epidemic of a relapsing fever occurring at Lwow in 1934 among persons employed in feeding laboratory reared lice to be used for the purpose of preparing Weigl's vaccine against classical typhus. Over the course of some weeks eighteen out of a personnel of twenty-four suffered from an illness, resembling trench fever excepting for minor clinical differences, which was found to be due to infection with a rickettsia transmitted by the lice. These organisms differed from those of epidemic typhus by reason of their greater size and pleomorphism, extracellular position and non-pathogenicity in the louse; furthermore, all the personnel had previously been vaccinated against typhus by Weigl's vaccine. Because of its pathogenicity for man MOSING believed the organism to be distinct from *R. pediculi* and stated that vaccines of *R. pediculi* did not protect against the new infection. With the new organism, which he called *R. weigli*, he prepared a vaccine which he suggested was efficacious, as among the six persons escaping infection four had been vaccinated and these were able to nourish lice infected with *R. weigli* without contracting the illness. HERZIG (1939) reported another outbreak in WEIGL's laboratory in 1939, and similar infections have been observed in Russia (YAKIMOFF, 1926), Japan (OGATA, 1935), France and Spain (SCHAPIRO, 1939). No opportunity has occurred, however, of studying vaccination against infections of the trench fever group on any large scale, but the experiments of MOSING would suggest that theoretically, vaccines could be prepared at least by Weigl's technique.

Q Fever.

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ANIGSTEIN (1933) in Malaya, cultivated from the blood of patients suffering from scrub typhus or from the tissues of infected animals on blood-agar, rickettsia-like organisms which he then suspended in saline containing 0·3 per cent. formalin. Using 200 and 400 million organisms at a week's interval, he vaccinated 300 coolies, on a highly infected oil-palm estate, and another seventy with only the first dose. During the first half of the year before vaccination there were forty-four cases, but in the second half of the year after vaccination there were only fifteen. Two cases occurred during the month of vaccination, but of the thirteen remaining, nine were among the unvaccinated, three among the vaccinated and one where it was uncertain whether vaccination had been done or not.

LEWTHWAITE (1939) was unable to confer any real immunity against tsutsugamushi fever to guineapigs by means of various vaccines prepared from

suspensions of *R. orientalis* treated with formol, phenol, egg yolk and olive oil, cysteine or combinations of cysteine and formol or phenol.

Trench Fever.

In view of the present war, it may be of importance to mention certain observations on trench fever and allied recurrent rickettsial diseases. During the 1914-18 War trench fever was according to BYAM (1919) responsible for a greater amount of sickness than any other infection on the Western Front, and the *Official History of the Great War* (1921) gives an estimate based upon the year 1917 that a force of a million would lose yearly by evacuation for trench fever some 45,000 men. Of these 80 per cent. would require 2 months at depots or bases and be off duty for 3 months, and of the remaining 9,000 about 2,000 would be incapacitated for more than 6 months. After the War trench fever unexpectedly disappeared before the question of vaccination had received any serious study, although BYAM states that an attempt had been made to vaccinate by means of an emulsion of louse excreta sterilized by heat or phenol.

MOSING (1936) described an interesting epidemic of a relapsing fever

The inoculation produces as a rule only slight local symptoms although occasionally there may be headache, some fever and general malaise for 24 to 48 hours ; rarely there may be an urticarial rash but such cases clear up without serious consequences. Protection varies greatly and, for this reason, yearly revaccination is recommended.

PARKER (1935) reported that only sixty-four out of 150,000 vaccinated in the endemic area developed spotted fever. Protection was complete against the mild Idaho virus, the morbidity falling among the highly exposed shepherds from 6 per cent. in the unvaccinated to 0·5 per cent. in the vaccinated, while against the highly virulent Bitter Root Valley virus of Montana, protection was shown by a fall in fatality from 82 per cent. among the unvaccinated to 6·6 per cent. among the vaccinated. Similar protection was given to laboratory workers handling the Bitter Root virus ; infection occurred in twenty-two cases, and of seven not vaccinated all were fatal, whereas among fifteen vaccinated only one died.

MONTEIRO (1933) showed that with the South American tick *Amblyomma cajennense* a similar vaccine could be prepared, effective in guineapigs, against North American spotted fever and Sao Paulo tick typhus, and CANET (1934) also produced a spotted fever vaccine using the European tick *Dermacentor reticulatus*.

OTHER INFECTIONS.

Tsutsugamushi Fever.

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MOSING (1936) described an interesting epidemic of a relapsing fever occurring at Lwow in 1934 among persons employed in feeding laboratory reared lice to be used for the purpose of preparing Weigl's vaccine against classical typhus. Over the course of some weeks eighteen out of a personnel of twenty-four suffered from an illness, resembling trench fever excepting for minor clinical differences, which was found to be due to infection with a rickettsia transmitted by the lice. These organisms differed from those of epidemic typhus by reason of their greater size and pleomorphism, extracellular position and non-pathogenicity in the louse ; furthermore, all the personnel had pre-viously been vaccinated against typhus by Weigl's vaccine. Because of its pathogenicity for man MOSING believed the organism to be distinct from R. pediculi and stated that vaccines of R. pediculi did not protect against the new infection. With the new organism, which he called R. weigli, he prepared a vaccine which he suggested was efficacious, as among the six persons escaping infection four had been vaccinated and these were able to nourish lice infected with R. weigli without contracting the illness. HERZIG (1939) reported another outbreak in WEIGL's laboratory in 1939, and similar infections have been observed in Russia (YAKIMOFF, 1926), Japan (OGATA, 1935), France and Spain (SCHAPIRO, 1939). No opportunity has occurred, however, of studying vaccination against infections of the trench fever group on any large scale, but the experiments of MOSING would suggest that theoretically, vaccines could be prepared at least by Weigl's technique.

Q Fever.

Immunity follows infection and can be demonstrated and utilized diagnosti-cally either by means of animal inoculations or by agglutination reactions with

suspensions of the specific rickettsia, but so far protective vaccination has not found any practical exploitation.

PASSIVE IMMUNIZATION.

Typhus Group.—From time to time the serum of convalescents has been used by many workers for the treatment of typhus, but LEVADITI and LÉPINE (1938) state that given during the course of the illness it neither arrests the evolution of the disease nor diminishes its gravity, an opinion confirmed by IONESCO-MIHAESTI and his colleagues (1939) in Roumania, although DURAND (1932), HELMAN (1934) and others believe they have observed beneficial results.

From the point of view of prophylaxis, however, convalescent serum may be of considerable service and, according to NICOLLE and CONSEIL (1920), has been useful in preventing infection after accidental contamination in the laboratory. The Experts of the League of Nations (1937) suggested that in case of a threatened epidemic of typhus, among the measures to be taken should be the formation of stocks of serum of immunized animals and the organization of a service for the obtaining of serum from convalescents. In the case of an epidemic they recommended the immediate use of immune sera in the first place, for the health and administrative personnel in contact with patients, with the exception of persons already immunized with killed vaccine more than 1 month and less than 12 months, and, in the second place, for persons having already come into contact with patients. WEIGL (1937) suggests that serum from those recently vaccinated may be used for the protection of contacts and has recently emphasized an interesting application of immune sera, either from convalescents or those vaccinated, in combination with vaccines, to which reference will be made later. PLAZY, GERMAIN and PLAZY (1932) believe they observed a favourable clinical action of serum from cases of murine typhus, not only against murine infection, but also against fièvre boutonneuse, and *vice-versa*. On the other hand, DECOURT and SALLARD (1930) in the case of typhus exanthematicus, found repeated prophylactic injections of convalescent serum failed to protect five doctors and nurses who developed severe infections, one being fatal ; they also found serum useless in declared cases.

The sera of horses or asses intravenously injected with increasing doses of infected tissues in spite of the early encouraging experimental results of NICOLLE and BLAIZOT (1916), CANTACUZÉNE CUICA, GALASECO and GÉRARD (1919) and others was abandoned in therapy until ZINSSER and CASTENADA (1933) again directed attention to such treatment. These last prepared a serum from horses by repeated inoculations with formolized suspensions of murine typhus from infected rats after irradiation. The serum showed neutralizing properties *in vitro*, agglutininated *B. proteus* X 19 to a titre of 1 : 320 as well as suspensions of *Rickettsia*, and protected guineapigs against infection with the homologous strain if given within 7 days before or 2 days after the infecting inoculation ;

partial protection was obtained against a heterologous strain of classical typhus by ZINSSER and CASTENADA, by VARELA, PARADA GAY and AGUAYO (1934) and by GIROUD (1935). ZIA and WU (1936) found that it protected guineapigs infected with a classical Peiping strain when administered 24 hours after the animals were infected and that some slight effect was produced when it was given 48 hours after the fever had started. Although it might not be curative in severe cases, encouraging results were obtained therapeutically in mild cases by VIESCA BENAVIDES (1933), and the Public Health Commission in Mexico (1935) and by BUSTAMENTE and his colleagues (1935) in Chile. By concentrating the serum, ZINSSER, CASTENADA and HAGER (1935) obtained greater potency and were able completely to protect guineapigs against either murine or classical virus.

Spotted Fever Group.—In Rocky Mountain spotted fever the serum of convalescents, given in the period of incubation prevented the development of experimental infections, according to NOGUCHI (1923) and SPENCER (1929), but antiserum has found no wide application in practical therapeutics. It is of historical interest to observe that, according to CANET (1934), Professor COOLEY wrote to RICKETTS in 1910 asking whether laboratory workers in Florence could be immunized by a serum against spotted fever, but RICKETTS did not propose to use his horse serum because, given repeatedly every 2 weeks as necessary, he had observed cases of anaphylaxis which decided him to give up the method.

The serum of patients recovered from fièvre boutonneuse has usually only a feebly protective action against the homologous virus and does not neutralize the virus of murine typhus (LÉPINE and CAMINOPETROS, 1932) ; yet, as already stated, PLAZY, GERMAIN and PLAZY (1932) reported a favourable clinical action of serum from patients recovered from either infection against either virus.

Tsutsugamushi Fever Group.—HAYASHI and his colleagues (1933) make a statement that anti-sera in combination with "a certain drug" gave satisfactory results against tsutsugamushi fever. LEWTHWAITE (1939) found the serum of a horse repeatedly injected with suspensions of *R. orientalis* failed to protect guineapigs although some very slight effect was observed when the serum was concentrated.

Summary.

It is suggested that, in any of the rickettsial infections, specific sera of convalescents or immunized animals are probably of considerable value if given immediately before contamination occurs or in the period of incubation of the disease, but that once the illness is manifest the therapeutic results are much less certain. For obvious reasons, therefore, the use of sera must tend to be confined to cases where infection is known or likely, as among laboratory workers who may suffer accidents, and among contacts of naturally occurring cases, especially of

epidemic typhus; in the prevention of the endemic fevers and sporadic infections immune sera naturally can receive little application. In treatment, the empirical use of specific immune serum would appear justifiable although so far scattered observations do not warrant much optimism for the therapeutic results.

SERO-VACCINATION.

Reference has already been made to the attempts of WEIL and BREINL (1923) to produce immunity by injection of mixtures of immune serum and infected material, and similar combinations have been used by FUKUDA (1929), ANIGSTEIN (1936), ZINSSER and MACCHIAVELLO (1936a) and others. Although it is possible to achieve active immunization in this way without any untoward reaction, the balance between the two agents needs to be accurately adjusted.

MARTINI (1919) used a mixture of DA ROCHA-LIMA's vaccine and serum from a horse immunized against typhus and thought the result was promising. WEIGL (1937) recently drew attention to the application of sero-vaccination. He pointed out that the phenolized louse vaccine administered during the incubation period of typhus does not protect, because the development of the virus outstrips the production of antibodies, but that if at the time of vaccination a dose of serum from a convalescent is also given, then an attack may be avoided. This would be a valuable treatment for contacts but there is the difficulty of getting sufficient supplies of convalescents' sera, especially at the beginning of epidemics. He found, however, that the sera of those vaccinated with his vaccine contain antibodies not only equal in concentration and protective power to those found in the most active sera of convalescents, but often even superior. He suggests therefore that the serum of persons recently vaccinated can replace that of convalescents.

RECENT DEVELOPMENTS.

Apart from the general interest of inducing immunity against rickettsial infections, the potential importance at the present time of possessing simple and practical methods of protection against typhus and trench fever requires no emphasis. Unfortunately, all methods of rickettsial vaccination so far described leave something to be desired. During recent years efforts have been made to obtain adequate suspensions from organisms grown in tissue culture or in the developing egg, and most recently by new methods of animal inoculation.

Tissue Culture Vaccines.—The early efforts of tissue culture met with only limited success but KLIGLER and ASCHNER (1934) prepared formolized vaccines of a Mediterranean murine virus and a classical European virus from cultures obtained using the technique adapted for rickettsias by NIGG and LANDSTEINER (1930 and 1932) by which the organisms were grown in minced guineapig tunica in serum-Tyrode solution, a method which enabled NIGG (1935 and 1936)

to maintain cultures of classical and murine virus over 3 and 4 years without any loss in virulence.

For the preparation of the vaccine the infected tissues were ground up in the culture-fluid and the suspension centrifuged at high speed for some time. After removal of the supernatant the deposit was triturated, twice frozen and thawed to break up the cells, again triturated and finally resuspended in the original supernatant or an equal volume of saline ; formalin was then added to 0·1 per cent. The vaccine is sterile and non-infective even in large doses ; in fact, the mere freezing and thawing seems sufficient to kill the virus. In guineapigs, three injections equivalent to one-sixth of a tunica produced immunity, some animals afterwards resisting 800 infective doses of virus.

GIROUD and PLOTZ (1935 and 1936) and GIROUD (1936) prepared attenuated vaccines according to the technique of NICOLLE and LAIGRET from material grown in such cultures.

With a view to modifying the method to give greater yields, ZINSSER with MACCHIAVELLO (1936b) and SCHOENBACH (1937) studied the physico-chemical requirements of rickettsia in tissue culture and came to the conclusion that the organisms multiplied most after metabolism of the tissues had slowed down and a certain equilibrium had been attained. At first a method similar to the previous one but on a larger scale was used, but later ZINSSER, WEI and FITZPATRICK (1937) developed a further technique in which tissue metabolic activity was held to a minimum under controlled conditions of pH and retarded autolytic change.

They used slants of a 4 per cent. agar solution in water mixed with an equal part of double strength Tyrode (later, 1939, containing 2 grammes sodium bicarbonate per litre) to which 50 per cent. horse serum was added before filtration ; phenol red was also added as an indicator, the pH being about 7·4 to 7·6. Minced guineapig tunica, infected by 10 to 15 minutes' contact with virus, was " buttered " on the agar and the cultures were closed excepting for a capillary outlet, controlled to allow CO_2 to accumulate or escape, so adjusting the reaction which is most important. Growth is apparent after 6 days and after 10 days smears are rich in rickettsias ; between these times transplants may be made. Large yields were obtained, the cultures were still infective after numerous passages, and formolized vaccines prepared from them immunized guineapigs.

It is possible to cultivate not only classical and murine strains but also the viruses of Rocky Mountain spotted fever and of Japanese river fever by such a method, so that theoretically the production of a vaccine against any of these viruses appears possible. It is also interesting to note that cultures in sealed tubes may remain virulent for long periods, a Mexican strain being found infective after 7½ months and a European strain after 4½ months ; one murine strain was so sealed and taken from America to China where it successfully infected animals. ZIA, PANG and LIU (1940) prepared a vaccine with agar slant cultures of classical virus suspended in 1 : 10,000 merthiolate and 0·5 per cent. phenol. After experiments with animals, 150 men were each given 2·5 c.c. of the vaccine in 3 doses at weekly intervals, with only slight local and insignificant general reactions. When tested a month later the majority of the vaccinated had developed increased Weil-Felix reactions.

The cultures can also be carried on using minced mouse embryo or chick embryo in place of guineapig tunica.

FITZPATRICK (1939) describes the production of vaccines with European virus and Rocky Mountain spotted fever virus grown for 7 to 9 days in minced mouse embryo on agar slants. The infected tissue was removed, ground up and suspended in 3 c.c. of 0·2 per cent. formolized saline per slant. (For human use she suggests light centrifugation at this point). After standing in the ice-box for a week to ensure the death of the organisms it was used to immunize monkeys and guineapigs. The animals developed positive Weil-Felix reactions and satisfactory immunity to subsequent test doses of virus.

Egg Culture Vaccines.—ZIA (1934) opened another line of research by showing that European or murine virus would grow on the chorio-allantois of the developing egg, but the yield was insufficient for the preparation of vaccines. Cox (1938) then found that viruses of Rocky Mountain spotted fever, murine typhus, classical typhus, fièvre boutonneuse, Brazilian spotted fever and an unidentified rickettsia from ticks in Texas would develop in the yolk sac of the developing chick embryo. The yolk sac suspensions are, as a rule, 100 to 1,000 times more infective than mammalian tissue or other tissues of the developing chick with murine typhus and Rocky Mountain spotted fever and, in the case of the latter virus, approach the limit reported for tick tissues. It would seem therefore that vaccines could be prepared from such material and Cox (1939 and 1940) has reported on the experimental production of such vaccines as well as vaccines prepared from the pooled tissues of the developing chick embryo.

Combined Culture Vaccines.—ZINSSER, PLOTZ and ENDERS (1940) have combined the yolk sac technique with their agar slant cultures using a considerably enlarged surface.

Macerated yolk sac on the 4th day of infection is used to inoculate normal minced chick embryo which is then spread on the agar surfaces. After 6 or 7 days' incubation at 37° C. cultures very rich in rickettsias are obtained, and the authors state that one bacteriologist and two technicians can produce in a week a litre of vaccine sufficient for 300 complete immunizations.

Lung-infection Vaccines.—Utilizing large concentrations of virus obtained by new processes of infection in animals other methods of preparing vaccines are possible. OKAMOTO (1937) found that after intraperitoneal inoculation of typhus into mice, rickettsias were found widespread in endothelial cells and that there were more infected cells in the lungs than in the liver or spleen, although WOHLRAB (1937) infecting etherized mice by the respiratory route reported neither pulmonary lesions nor any particular abundance of rickettsias in the lungs. CASTENADA (1939) however recently found intranasal instillation of murine virus into etherized rats or mice produces a pneumonia characterized by considerable development of rickettsias, and that similar lesions can be produced in rabbits but to a lesser degree unless the temperature of the animals is artificially lowered. DURAND and SPARROW (1940) confirm these findings, remarking that despite the eclectic development of rickettsias in endothelial cells hardly anyone has sought to cultivate the organisms in the lung although it is so rich in such cells. They find the animals of choice are white mice which, after infection by the respiratory route with virulent

material, die in 3 to 6 days with a progressive hypothermia. The lungs are practically completely affected by a haemorrhagic hepatization and animals of passage inoculated with 1 : 10 to 1 : 100 of such lungs die in 40 to 96 hours, while smears from their lungs show enormous quantities of free rickettsias, probably from ruptured cells ; a ten-thousandth of the lungs will kill a rat. By grinding up the lungs and by differential centrifugation, it is easy to obtain from the lungs of one mouse 10 to 20 c.c. of an almost cell-free suspension of rickettsias corresponding in opacity to a suspension of 1,000 million typhoid organisms per c.c. The rat and the rabbit have given results inferior to that with the mouse.

Classical virus produces almost nothing if inoculated in the form of emulsions of infected guineapig tissues. On the other hand, the product obtained by grinding up the intestines of one or two infected lice kills the mice in 3 days, further passages often producing death in less than 40 hours. The pulmonary lesions are as intense and rich in rickettsias as those produced by murine typhus.

The grey domestic mouse, the field mouse (*Mus spretus*), the striped rat (*Mus barbarus*) react as the white mouse ; the jerboa and-gerbil almost the same but the " Mérion " (? *Meriones*), white rat and guineapig progressively less so, while the rabbit yields few rickettsias.

With either type of virus rickettsias are not found easily excepting in the lungs and pleura, although the brain and spleen of mice are virulent. The virus of fièvre boutonneuse with the same technique behaves similarly in the white mouse and merion ; it is probable according to DURAND and SPARROW that this will hold for the other exanthematic viruses.

This important advance provides a simple and rapid method for obtaining rich emulsion of typhus rickettsias and its potential application to vaccination is obvious.

DURAND and GIROUD (1940) have already prepared a formolized vaccine against classical typhus by this method.

The lungs of mice (and eventually of rabbits) infected by the respiratory route were ground up and emulsified in human or equine serum diluted 1 : 5 in physiological saline containing 2 per cent. formalin. By fractionating centrifugation the cells and cellular débris were removed and the vaccine then stored for at least 5 days in the ice chest before being used.

Preliminary tests in guineapigs and monkeys having given satisfactory results the authors then made trials in man.

Twelve subjects were each given in three subcutaneous injections at weekly intervals doses of vaccine corresponding to about 7/100, 12/100 and 18/100 of mouse lung. A few showed a slight reaction with temperature of 100·4° F. to the first injection but nothing to those following.

The Weil-Felix reaction with OX 19, negative in the beginning, became positive in all cases, frequently to considerable titres, from the 8th day after the

second injection, and for the most part even more elevated 18 to 21 days after the third injection ; the agglutination reaction with *R. prowazeki* showed a similar progression.

The sera of eleven out of the twelve vaccinated, tested by GIROUD's (1938) cutaneous serum-protection test, reacted as does the serum of typhus convalescents neutralizing virus completely or almost so ; the serum of the twelfth gave a paradoxical reaction, the cutaneous lesion in the rabbit being more intense with the patient's serum and virus mixture than with control normal serum and virus mixture. Sera of some of those vaccinated showed a slight neutralization with murine virus.

Further vaccination trials were made using doses about three times less than the previous, obtained from either mouse or rabbit lungs and given at 5-day intervals. Similar results were obtained.

It remains to be seen whether the trench fever virus and the other viruses will develop in the same way in suitable animals, so that the practical outlook for a vaccine against such infections is for the moment obscure.

SUMMARY.

1. Recovery from a rickettsial infection is accompanied by the development of considerable immunity ; therefore, protective vaccination is theoretically possible.

2. In practice, difficulties are encountered because living vaccines may be dangerous, and dead rickettsias have relatively little immunizing power.

3. These difficulties might be overcome by using vaccines of living but attenuated organisms or relatively large numbers of killed organisms.

4. Large numbers of organisms are not conveniently obtainable as rickettsias will not grow in ordinary media. They can, however, multiply enormously in appropriate arthropods, and PARKER and SPENCER utilized this to prepare a satisfactory killed vaccine against Rocky Mountain spotted fever from phenol-formolized suspensions of ground-up infected ticks.

5. WEIGL prepared an effective typhus vaccine by emulsifying in phenol-saline the heavily infected intestines of artificially infected lice. By Weigl's technique, MOSING prepared a specific vaccine which he thought had some action against a trench fever-like disease. The labour involved limits production and Weigl's vaccine has also been criticized on the general ground that killed vaccines only give feeble, restricted and short-lived immunity.

6. Infections can also be developed in laboratory animals and early attempts

were made to use infected tissue suspensions as vaccines, but the small yields of rickettsias when killed failed to vaccinate satisfactorily.

7. As living organisms, although attenuated, might give rise to accidental infections, the danger in typhus was diminished by using the relatively benign murine virus which, owing to cross immunity between it and classical typhus, can when living immunize against either infection. Although considerable cross immunity exists between classical and murine typhus, the enfeebled protective power of killed rickettsias is manifest in a much stricter specificity of the respective killed vaccines in the two diseases.

8. BLANC and his colleagues rendered living murine virus from infected guineapigs innocuous by treating it with ox-bile ; the virus is very unstable and a modification has been introduced whereby dried infected flea faeces, which retain their virulence for long periods, are used as the source of virus for the vaccine.   NICOLLE and LAIGRET attenuated a murine virus from guineapigs and rats by drying and coating it with egg yolk and olive oil ; LAIGRET and DURAND have introduced a further modification using mouse-brain passage virus. These vaccines normally produce only inapparent infections, reactions of vaccinial typhus being rare.   Nevertheless, in certain circumstances, such reactions may not be negligible.

9. ZINSSER, disbelieving the reliability of controlled attenuation, attempted to obtain sufficient quantities of rickettsias for killed vaccines from animals heavily infected by having their resistance artificially lowered.

10. Thus ZINSSER and CASTENADA, with murine virus in rats irradiated with X-rays, obtained peritoneal accumulations of virus sufficiently rich to prepare a practical phenolized vaccine.   Its protection against classical typhus was, however, poor, nor could they get satisfactory yields of classical virus from irradiated rats.

11. Efforts are now being made to produce vaccines of various rickettsias grown in tissue cultures, and in the yolk sac and tissues of developing chicks ; other experiments seek to obtain sufficient yields from animals by simpler methods.   DURAND and SPARROW recently reported an important technical advance whereby intranasal instillation of typhus viruses into etherized white mice results in a rapid and massive pulmonary infection, from which rich cell-free suspensions of rickettsias can be prepared. Utilizing this, DURAND and GIROUD have recently prepared mouse lung vaccines against classical typhus.

12. Sera from convalescents, from those vaccinated or from animals artificially immunized, may confer good but brief passive immunity against rickettsial diseases ; the curative action in declared infections is less certain. Combined sero-vaccination has been suggested to give typhus contacts immediate protection followed by active immunity.

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DISCUSSION.

Dr. G. M. Findlay : Mr. President, ladies and gentlemen, I recently had the privilege of spending some weeks in North Africa and a short time in Paris, where I had an opportunity of seeing much of the recent work on immunization against typhus. As a result of that trip, and possibly also because I have just recovered from an attack of murine typhus myself, my views on much of the work which Dr. Murgatroyd has described are not so rosy, perhaps, as those his paper would suggest. As he pointed out there are two general methods in use for immunizing against classical typhus: they are the use of the murine strain of rickettsiae alive or attenuated, and the use of killed exanthematic rickettsiae. With regard to the use of the murine strain it may be said that Laigret and Robert Durand have produced a vaccine which is by no means attenuated: that is to say, it will kill mice with very extensive lesions in the brain. The egg yolk vaccine and the previous modification, which was made in olive oil, have been given to two million natives in Tunis, approximately one tenth of the population. There are said to have been practically no reactions to these injections. Typhus epidemics have been brought to an end, and where immunization has been carried out there has been no recurrence of the epidemic for some few years. Nevertheless, despite the fact that two million people have been inoculated, there have been no scientifically controlled experiments to prove the value of this method. Vaccination has been carried out thoroughly only in face of actual epidemics of typhus in villages in the country; but as one knows, having learned it in the last war, native populations hide as long as possible the fact that a typhus epidemic is occurring, and therefore it is probable that a considerable number of natives who are inoculated are already immune to typhus.

immunized against murine typhus but who seemed to have a typical attack of murine typhus. A point in connection with immunization against typhus is that immunizing with the living murine typhus will not protect against exanthematic typhus during the incubation period of the latter. One case that I saw developed high temperatures 8 days after inoculation with the murine typhus, and the exanthematic virus was obtained from his blood.

With regard to BLANC's bile-treated vaccine; the original preparation was, of course, extremely unstable. If the preparation was made in the morning with a saline preparation of guineapig's tissue mixed with ox bile it was probably dead in the afternoon. That, as Dr. MURGATROYD has pointed out, has now been modified by the use of the faeces of fleas fed on infected rats, and containing the murine virus. It is quite possible that the bile may attenuate some of the rickettsiae, possibly killing them, but leaving their antigenicity. That point needs to be further worked out. Again although there are said to be no reactions in the native Moroccans who have been subjected to this particular treatment, the reactions in certain Europeans are quite severe. The Director of Hygiene in Morocco told me that of Europeans some 30 per cent. have severe febrile reactions: the use of bile-treated murine virus is therefore at present hardly practicable for Europeans, whatever it may be for natives.

We now come to killed viruses. The original method was that of Weigl, in which lice are inoculated *per rectum*. An expert can inoculate from 40 to 50 lice per hour, but it is not an easy technique to learn, and apart from the difficulty of inoculation there is a further difficulty in making the vaccine: the lice during the process of incubating the typhus must be nourished on people already immune to typhus. These hosts have to be changed about every three or four weeks, because they become anaemic, from the large number of lice fed on them, and one has to be careful in choosing these nourrisseurs that they do not infect the lice with rickettsiae of the Da Rocha Lima type which develop extra-cellularly and intra-cellularly in the louse intestine; if the louse becomes infected with rickettsiae of the Da Rocha Lima type it cannot also develop the exanthematic rickettsia. Rickettsiae of the Da Rocha Lima type are non-pathogenic to man, and there is no evidence that any Europeans have immune bodies against them. They are picked up from the skins of Europeans by lice when feeding.

Finally as to other methods. There is the egg method of tissue culture and the method of agar slants. Inoculation into the yolk sac of eggs gives a rich culture of virus rickettsiae but no actual immunization of human beings has been carried out by egg culture. In China ZIA and his colleagues have employed the agar slant method of ZINSSER, and inoculated people who subsequently developed positive Weil-Felix reactions. The method which I saw being practised in the Pasteur Institute in Tunis is that which PAUL DURAND and GIROUD have worked out, and consists of the intra-nasal instillation of the exanthematic typhus rickettsiae under ether anaesthesia into mice. The

mice must be small, not more than 20 grammes each, and preferably of the Swiss strain. When the rickettsiae have developed after a few passages the mice die in 48 hours, not with hyperthermia, but with a hypothermia. The temperatures of the mice must be very carefully watched, and to get the maximum yield of rickettsiae from these mice they should be killed when the temperature comes down to about 32° C. to 30° C. This yields a very rich harvest of rickettsiae, and by differential centrifugation a suspension practically free from tissue can be obtained. I myself experienced no reaction when immunized by this method and I developed a positive Weil-Felix and also rickettsiacidal properties in the blood, the presence of which can be shown by the intradermal rabbit test of Durand. Previously Dr. MURGATROYD had immunized me by the Weigl method with what was equivalent to 162 louse intestines, but presumably the vaccine was old, because I did not develop either a positive Weil-Felix or rickettsial immune bodies. Mouse lung vaccine can keep its immunizing power for at least six months if kept in the cold, and although possibly mice are the most suitable animals for producing small quantities of vaccine, Dr. GIROUD informs me that he has produced very large quantities of vaccine from rabbit lung. If a rickettsial suspension is inoculated intratracheally into rabbits a very intense rickettsial pneumonia develops which is again swarming with organisms. How long the immunity lasts is not at present known, but it certainly persists for some months. As Dr. MURGATROYD pointed out killed exanthematic rickettsiae do not necessarily give immunity against murine typhus, but I certainly think they give some immunity because I have just had what is apparently a mild attack of murine typhus. My temperature went up to 101° F. and I only had 5 days' fever; whereas one of my assistants who had not been immunized against classical typhus had quite a typical attack of murine typhus with temperatures of between 103 and 104° F. Inoculation with killed exanthematic rickettsiae may not therefore give complete immunity against murine typhus but it certainly appears to give some degree of immunity.

As regards trench fever no effort has yet been made to produce a vaccine, but there seems to be no reason why it should not be made if necessary, employing some of the methods which have now been worked out for making killed vaccines by the growth of tissue culture or growth in chick embryos.

Miss D. Lush : Actually we have not done any work in Australia from the point of view of human immunization at all. All I can say is that the bandicoot is definitely known to be one of the reservoirs, at least, in Queensland. A tick, *Haemaphysalis humerosa*, that infests it is known to sometimes carry the rickettsiae of Q fever and attack animals, cattle and horses. It is not known to attack man but under experimental conditions it did so on one occasion. No attempt has been made to do vaccinating. I think, because it is a very mild disease as it occurs in Australia.

The American Montana disease, now called American Q fever, is spread by the tick, *Dermacentor andersoni*, and we can find no antigenic difference between the two strains of disease; but it is undoubtedly more virulent than Australian Q fever in animals.

The President, Sir RICKARD CHRISTOPHERS, said it was often stated that it was possible to become infected with a louse-borne disease through crushing the lice when scratching. He wondered whether it was really possible to crush lice so easily by the act of scratching.

Dr. Murgatroyd (in reply): Scratching could make abrasions through which typhus infection might enter, but whether. as is commonly believed, it was actually possible by scratching to crush lice he did not know. Even if crushing were impossible might not scratching perhaps squeeze faeces out of the lice?

He sympathized with Dr. FINDLAY's present feelings but hoped the paper represented an objective view of the position neither unduly rosy nor unduly jaundiced.

COMMUNICATIONS.

CONCENTRATION OF BAYER 205 (GERMANIN) IN HUMAN BLOOD AND CEREBROSPINAL FLUID AFTER TREATMENT.

BY

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The present paper describes an investigation into the concentration of Bayer 205 (germanin) found in the blood of sleeping-sickness patients who had been treated with this compound. The work was carried out at Kahama in Tanganyika Territory, East Africa, which is the centre of an area of endemic Rhodesian sleeping-sickness. A few patients were examined also at the Gadau Laboratory of the Nigerian Sleeping Sickness Service. The concentration of Bayer 205 was estimated by the method of DANGERFIELD, GAUNT and WORMALL (1938).

Briefly, the technique was as follows: 3 ml. of the citrated plasma was heated to 100° C. for six hours with 3 ml. concentrated hydrochloric acid. It was made up to 10 ml. with water, about 3 grammes of kaolin was added, and the mixture was filtered. Two ml. of the filtrate was treated successively with 1 drop 0·5 per cent. sodium nitrite, 2 drops saturated urea, 3 ml. 30 per cent. sodium acetate, and 1 ml. 0·2 per cent. mono-methylnaphthylamine hydrochloride in 50 per cent. acetic acid. A red colour develops, which was matched against standard coloured discs in a Lovibond comparator. In obtaining specimens, the blood was citrated and soon afterwards centrifuged to obtain the plasma; 3 ml. of the plasma was immediately mixed with 3 ml. of the concentrated hydrochloric acid. In some cases the samples were then stored in the tubes for a day or two before heating in the boiling water bath. This practice is convenient but it may lead to an increase in the apparent amount of Bayer 205 observed: when the effect of standing was investigated by making up

samples in duplicate, one being estimated at once and the other stored for seven days, an increase of 20 to 40 per cent. was observed.

Normal Controls.

By this method of estimating Bayer 205, normal plasma gives a small blank value presumably due to amines originally present or produced by the hydrolysis. A series of determinations was therefore made on patients who had received no drug and the results are shown in Table I.

TABLE I.

SHOWING THE BLANK VALUES (EXPRESSED AS EQUIVALENT AMOUNTS OF BAYER 205) OBTAINED IN THE EXAMINATION OF PLASMA FROM UNTREATED PATIENTS.

Patient.	Sex.	Age Years.	Weight kg.	Blank Value. (Equivalent concentrations of Bayer 205) mg. per 100 ml.
Mitaki	M	25	59	1·0
Maganda	M	50	? 50	0·7
Mwizalawi	F	25	32	0·9
Georgi	M	20	34	1·0
Nyakmezi	F	22	49	0·8
Shivaheye	M	? 30	—	0·7
Shiluna	M	? 30	—	0·8
Ngala	M	? 25	—	0·9
Solce	M	? 35	—	0·8
Kube	M	? 35	—	0·9
Msuhanyala ...	M	45	53	1·0 / 0·9
Damiana	M	? 25	—	0·8
Mlisho	M	20	47	0·8
Bundala Nyamiti —	M	32	54	0·6
Dejia*	M	? 35	—	0·8
Mano Birinkudu* ...	M	? 35	—	0·8

*Nigerian case.

Average blank value, 0·84.   Range, 0·6–1·0.

These results show that the blank value ranges from 0·6 to 1·0 mg. per 100 ml., with an average of 0·84 mg. per 100 ml., i.e., a colour is obtained equivalent to 0·84 mg. of Bayer 205 in 100 ml. This is practically identical with the blank value obtained by DANGERFIELD, GAUNT and WORMALL in rabbits, viz., 0·8 to 0·9 mg. per 100 ml.; and the plasmas of two untreated rabbits, examined here for comparison, gave blank values of 0·7 and 0·9 mg. per 100 ml. According to VIERTHALER and BOSELLI (1939) the blank value obtained from

one and the same animal varies on different days, one rabbit ranging from 0·9 to 1·65 mg. per 100 ml. in their experiments. In all subsequent results concerning patients who had received Bayer 205, the average blank value (0·84 mg.) has been subtracted from the observed reading. Whenever possible, a reading was obtained of the blank value of the patient's own plasma before the treatment began. But in view of the fluctuation observed by VIERTHALER and BOSELLI in the same individual, it was considered safer to apply only this

TABLE II.

SHOWING THE CONCENTRATION OF BAYER 205 IN THE PLASMA OF PATIENTS WHO HAD RECEIVED A SINGLE I GRAMME DOSE OF THIS COMPOUND INJECTED INTRAVENOUSLY.

Patient.	Sex.	Age Years.	Weight kg.	Interval Days.	Concentration of Bayer 205 mg. per 100 ml.
Ntabasigaye ...	M	25	53	0·75	5·8
Johana ...	M	25	? 55	0·9	5·2
Masalu Manyenye	F	30	46	1	3·5
Buneya ...	M	25	51	1·7	4·2
Ngalo	M	25	48	1·7	2·8
Mahinga ...	M	25	51	1·7	2·8
Juma Saindi ...	M	40	? 55	1·75	3·5
Ntabasigaye ...	M	25	53	3·75	1·2
Buneye	M	25	51	4·75	2·2
Ngalo	M	25	48	4·75	0·8
Mahinga ...	M	25	51	4·75	1·2
Johana	M	25	? 55	7·25	1·0
Ntabasigaye ...	M	25	53	7·75	1·2
Buneya	M	25	51	8·75	0·65
Ngalo	M	25	48	8·75	0·5
Mahinga	M	25	51	8·75	0·65
Ngamilo... ...	M	45	52	{ 86 / 101	0·5 / 0·8

one standard correction in all cases. Since the blank value ranges from 0·6 to 1·0 mg., no significance can be attached to readings only 0·2 to 0·3 mg. higher than the average blank.

Concentration in Blood after a Single Dose.

Determinations were made on the blood of patients who had received a single intravenous injection of 1 gramme of Bayer 205, no allowance being for the differing weighs of the patients who were all adults. The results are shown in Table II and Figure 1: the latter also contains the readings obtained after the first dose from patients who received a series of injections as described in the next section of the paper. There is considerable difference in the

figures obtained from different individuals, much greater than can be explained by the difference in their body weights. Theoretically, in an ordinary person whose blood volume could be assumed as 5 litres, a dose of 1 gramme would produce a concentration in the blood of 20 mg. per 100 ml. The average of the five estimations done 18 to 24 hours afterwards (Fig. 1) is only 4 mg. so that about 80 per cent. of the compound leaves the blood within the first day. This is a large proportion, but not so large as that which occurs with most other compounds injected into the blood; e.g., when 3 grammes of tryparsamide are injected intravenously into man, about 95 per cent. disappears within
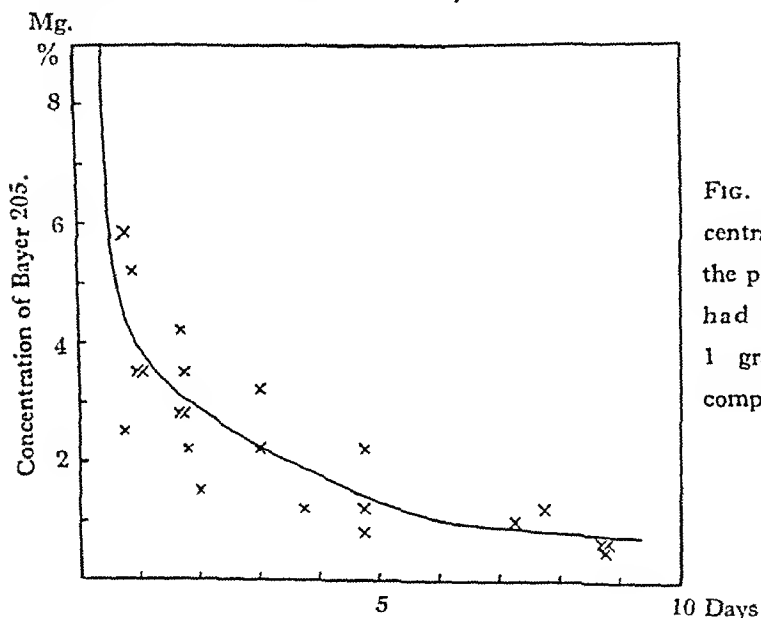
Fig. 1.—Showing the concentration of Bayer 205 in the plasma of patients who had received a single 1 gramme dose of this compound injected intravenously.

24 hours. Further details of this will be given in a later paper. After nine days, the concentration has fallen to 0.6 mg. per 100 ml., which is not greatly above the range of variation for the blank reading. It was not possible to leave patients for more than nine days after a single dose without proceeding to further treatment of some kind or other. In a single case, *Ngamilo*, the man ran away and returned three months later, but the results obtained from his blood should be regarded with caution, especially as there is a discrepancy between the two readings; possibly they represent nothing more than unusually high figures for the blank value. The amounts here found in man after 3 to 4 days (about 1.7 mg. per 100 ml.) are less than those found by DANGERFIELD, GAUNT and WORMALL (1938) in rabbits which had received a single dose of 28 mg. per kg. (1.4 gramme for a 50 kg. man): WORMALL *et al.* found 3.6 mg. after four days. In two rabbits injected by the writer for comparison, with 28 mg. per kg. and examined after 3¾ days, the amount found

in the plasma was 6·5 mg. per cent. in both cases. VIERTHALER and BOSELLI (1939) found 1·4 mg. per 100 ml. after four days in rabbits which had received 4·5 mg. per kg., *i.e.*, 0·22 gramme for a 50 kg. man. Apparently man retains the compound in smaller amounts than do rabbits.

Concentration in Blood during a Course of Four Doses.

Nine patients were given a course of four 1 gramme doses at various intervals. Just before giving each dose, a sample of blood was withdrawn for
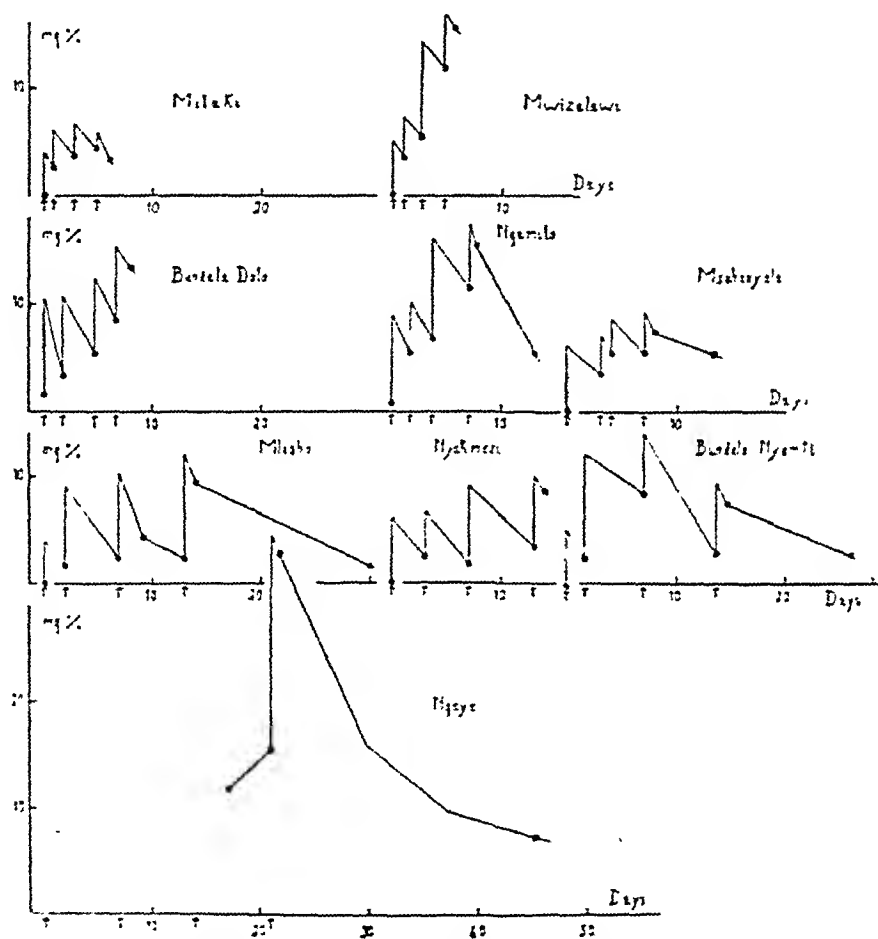
FIG. 2.—Showing the concentration of Bayer 205 in the plasma of patients, while they were receiving four 1 gramme doses of this compound.
The time of each injection is indicated by arrow.

examination and samples were also collected at other convenient intervals. The results are shown in Table III, and are reproduced graphically in Fig. II. They confirm the finding of the preceding paragraph, that there is a great difference between various individuals as regards the concentration of Bayer 205 reached in the blood by these procedures. Although naturally the shorter the

TABLE III.

SHOWING THE CONCENTRATION OF BAYER 205 IN THE PLASMA OF PATIENTS WHO RECEIVED 4 DOSES OF THIS COMPOUND, EACH DOSE BEING 1 GRAMME INJECTED INTRAVENOUSLY.

Patient.	Time Days.	Injections.	Concentration of Bayer 205, mg. per 100 ml.	Urine: protein mg. per 100 ml.	Remarks.
Mitaki Male, 25 years, 59 kg.	4 doses by 5th day. 0		0·15	0	
		1st			
	0·75		2·5		
		2nd			
	2·75		3·8		
		3rd			
	4·8		4·2	0	
		4th			
	6		3·8		
Mwizalawi Female, 25 years, 32 kg.	0		0	0	
		1st			
	1		3·5		
		2nd			
	2·75		5·2		
		3rd			
	4·75		11·8	10	
		4th			
	5·6		15·2	24	
Bundula Dolo Male, 25 years, ? 50 kg.	4 doses by 7th day. 0		1·5	Trace	Had received 7·5 grammes 9 months previous
		1st			
	0·75		7·2		
	1·8		3·2		
		2nd			
	4·8		5·2		
		3rd			
	6·8		8·2		
		4th			
	8		13·2		
Ngamilo Male, 48 years, 51 kg.	—		0·5		86 days after 1 gramme
	0		0·8		101 „ „ „
		1st			
	1·8		5·2		
		2nd			
	3·8		6·5		
		3rd			
	7		11·2		
		4th			
	7·8		15·2		
	13		5·2 ?		Technique unsatisfactory

TABLE III—*continued*.

Patient.	Time Days.	Injections.	Concentration of Bayer 205, mg. per 100 ml.	Urine : protein mg. per 100 ml.	Remarks.
Msuhanyala Male, 45 years, 53 kg.	0		0	0	
	3	1st	3·2	Trace	
	4	2nd	5·2		
	7	3rd	5·2	0	
	8	4th	7·2		
	13·3		5·2		
	4 doses by	14th day			
Mlisho Male, 20 years, 47 kg.	0		0		
	2	1st	1·5		
	7	2nd	2·2		
	9·2	3rd	4·5		
	13		2·2		
	14	4th	9·2		
	30·2		1·8		
Nyakmezi Female, 22 years, 49 kg.	0		0		No albumin 30 days previous
	3	1st	2·2	34	
	7	2nd	1·8	0	
	13	3rd	3·2	80	
	13·9	4th	8·5	70	
Bundala Nyamiti Male, 32 years, 54 kg.	0		−0·2*		
	1·8	1st	2·2		
	7	2nd	8·2		
	9	3rd	3·2 ?		Technique unsatisfactory
	13·75		2·8		
	14·7	4th	7·2		
	28		2·8		
	4 doses at	weekly intervals.			
Ngaye Female, 20 years, 36 kg.	0	1st	—		Had received 1 gramme 70 days previous
	7	2nd	—		
	14	3rd	—		
	17		11·8		No injection given in this interval
	18			44	
	21	4th	15·2		
	21·7		33·8		
	45		7·2		

*The actual reading in this case was 0·6 mg. per 100 ml., which is 0·2 mg. *less* than the average blank value (0·84 mg.) which has been subtracted from all the readings.

period within which the doses are given, the higher the final concentration in the blood tends to be, yet the concentration reached seems to depend quite as much on the individual peculiarities of the patient as on the spacing of the doses. The patient *Ngaye*, came under observation when her course of treatment was already partially complete, but the results are so remarkable as to deserve comment. Although there is a slight discrepancy between the first two readings obtained, all the four estimations show amounts much greater than would have been expected from experience with other patients: apparently she had a special tendency to retain the compound in her blood. At the other extreme is the case, *Mitaki*, in whom retention was much less than usual. Mention may also be made here of three patients (to be described more fully in a later paper) who relapsed during or shortly after a course of Bayer 205 and in whom the concentrations in the blood were remarkably low, *e.g.*, nil, two days after the 3rd dose; 1·0 mg. per 100 ml., five days after the 3rd dose; 4·2 mg. per 100 ml., two days after the 7th dose. Presumably these differences are due to the differing rates at which individuals are able to excrete, divert, or decompose the compound. The figures for the amount of protein in the urine were obtained by precipitating with trichloracetic acid in a Sicard and Cantaloube tube as for cerebrospinal fluid: unfortunately they are too incomplete to permit any conclusion to be drawn.

Concentration in Blood following 3 to 7 doses.

Estimations were also made on patients who had received courses of treatment before they were seen by the writer. These injections had been given by the Sub-Assistant Surgeon at Kahama, or by trained Africans at outlying dispensaries. Although the accuracy of these records cannot be guaranteed in every instance, yet whenever they could be checked by the patient's statement they were found correct, and there seems no reason to doubt their general reliability. Many of these courses are irregular, owing to alternation with tryparsamide, which is not shown, and to other causes, and the observations over the shorter periods are not always properly comparable; but they show the amounts which are actually obtained in ordinary hospital practice.

The results are given in Table IV, and they are shown graphically in Figs. 3 and 4, which also contain the final readings after the courses of four doses which have been described individually above. Here again, there are marked differences between the various individuals. Judging by these findings when four doses have been given, the compound cannot be demonstrated in the blood after about 230 days, but after five or six doses have been given, it can often be demonstrated after 250 days: in some patients, however, it disappears within 150 days or less. In one patient (*Bundala Dolo*), who had received four doses totalling 7·5 grammes, an estimation of the plasma-content showed 1·5 mg. per 100 ml. after 21 months.

FIG. 3.—Showing the concentration of Bayer 205 in the plasma of patients who had received four 1 gramme doses of this compound.

FIG. 4.—Showing the concentration of Bayer 205 in the plasma of patients who had received five to seven 1 gramme doses of this compound.
× indicates patients with 5 doses.
● „ „ 6 „
□ „ „ 7 „

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●   ,,   ,,   6 ,,
□   ,,   ,,   7 ,,

Concentration in Cerebrospinal Fluid.

Estimations were made on samples of cerebrospinal fluid obtained from patients who had received the drug at various preceding intervals, and the readings were compared with those from samples of the plasma taken simul-

TABLE IV.

SHOWING THE CONCENTRATION OF BAYER 205 IN THE PLASMA OF PATIENTS WHO HAD RECEIVED 3 TO 7 DOSES OF THIS COMPOUND, EACH DOSE BEING 1 GRAMME INJECTED INTRAVENOUSLY.

Patient.	Sex.	Age Years.	Weight kg.	Days since Doses.	Concentration of Bayer 205 mg. per 100 ml.
				3 doses	
Masiku Kidutu	M	40	52	149, 152, 154	−0·2†
Sija	M	30	—	55, 59, 62	1·0
				4 doses	
Malilo	M	50	45	1·75, 9, 16, 22	6·2
*Gezare**	M	? 35	67	4, 18, 23, 28	3·2
Kahorwe	M	22	50	4, 53, 60, 107	5·2
*Shehu**	M	? 35	63	104, 106, 111, 114	0
Ntimba	M	40	49	121, 128, 135, 141	0·8
Mlesi	M	35	62	141, 150, 157, 164	1·8
Mihayo	M	30	57 {	146, 152, 154, 156	1·0
				282, 288, 290, 292	0·
Mugulala	M	35	57	229, 254, 261, 264	0
Buchanagandi	M	? 35	50	233, 240, 254, 261	0·5
Juma Maganga	M	35	56	282, 290, 292, 296	0
				5 doses	
Mayombo	M	33	? 50	22, 29, 36, 43, 97	0·15
Ngili	M	50	54	69, 76, 97, 110, 117	6·5
Nawile	F	30	? 50	76, 83, 96, 103, 151	2·2
Kaseela	M	30	64	84, 91, 97, 102, 110	2·2
*Momo**	M	? 35	? 55	117, 122, 127, 131, 136	0·15
Mwikamwia	M	25	? 55	128, 135, 142, 149, 188	0·65
Kafuku	M	12	35	142, 156, 161, 170, 204	2·5
Mbiho	M	31	62	154, 161, 170, 177, 211	1·0
Fungameza	M	? 30	? 50	164, 171, 178, 187, 221	0·15
Nangoma	F	45	48	219, 233, 261, 267, 306	0·65
Rubunga	M	18	28	259, 286, 309, 314, 367	0·3
Bundala Dolo	M	25	? 50	644 (2·5 g.), 658 (2 g.), 665, 670, 672	1·5

TABLE IV—*continued*.

Patient.	Sex.	Age Years.	Weight kg.	Days since Doses.	Concentration of Bayer 205 mg. per 100 ml.
				6 doses	
Maziku Mupalage	M	40	44	19, 26, 35, 42, 47, 50	7·5
Nakubigwa	F	32	? 40	39, 46, 53, 60, 67, 74 ; 4 × 1 g., 260-320 days	6·2
Bundala Matwiga	M	35	? 55	41, 48, 55, 62, 69, 76	4·2
*Gambo**	M	? 35	50	60, 64, 68, 71, 74, 78	0·8
Budada	M	30	80	85, 92, 99, 106, 140, 143	1·8
Salim	M	32	52	89, 96, 103, 106, 108, 127	4·2
Katikilo	M	40	55	251, 345, 365, 407, 434, 462	1·5
Musasa	M	38	? 55	252, 259, 266, 273, 280, 283	0·15
Mbiho	M	23	60	261, 267, 383, 408, 441, 469	1·2
				7 doses	
Maziku Nkandi	M	40	45	1·7, 11, 84 (2 g.), 98, 105, 111, 122	12·5
Ndumbagwe	F	11	32	3, 10, 17, 24, 56 (¾), 70 (¾), 79 (½)	17·2
*Garuba**	M	—	—	7, 12, 110, 115, 120, 125, 130	4·2
*Saidu**	M	? 35	50	42, 45, 63, 69, 70, 71, 73	0·8

*Nigerian case.

† The actual reading in this case was 0·6 mg. per 100 ml., which is 0·2 mg. *less* than the average blank value (0·84 mg.) which has been subtracted from all the readings.

taneously. The results are shown in Table V. In the column referring to the cerebrospinal fluid, it is stated that a " very faint trace " was usually observed: this requires amplification. As was described at the beginning of this paper, the Wormall test for Bayer 205, when done on normal plasma, yields a blank value equivalent to about 0·8 mg. per 100 ml. of the drug: and in all figures relating to the plasma, this amount has been subtracted from the actual readings obtained. In the case of cerebrospinal fluid from untreated patients, no blank value is obtained (cf. the first two cases in Table V). With most of the samples from treated patients it was found that no appreciable colour could be observed by ordinary methods: but when the filtrates, treated in the usual way, were compared with filtrates to which all the reagents except the one drop of sodium nitrite had been added, a slight difference could be detected, *viz.* that, although the sample was still practically colourless, it had a slightly *duller* tint than that of the control. In one case (*Bundala Dolo*) in which this was marked, an attempt was made to obtain a quantitative interpretation by

comparing it with standards made from dilute solutions of Bayer 205. The standard corresponding to an amount of 0·03 mg. per 100 ml. gave the nearest approximation to the cerebrospinal fluid, but the match of colour was not very good, the cerebrospinal fluid preparation being rather yellowish while the standard was pinker and clearer; and only slight significance can be attached

TABLE V.

COMPARING THE CONCENTRATIONS OF BAYER 205 IN THE CEREBROSPINAL FLUID AND PLASMA RESPECTIVELY OF PATIENTS WHO HAD BEEN TREATED WITH THIS COMPOUND IN 1 GRAMME DOSES.

Patient.	Sex.	Age Years.	Weight kg.	Cerebrospinal Fluid.			Days since Doses.	Concentration of Bayer 205 mg. per 100 ml.	
				Trypanosomes.	Cells per mm.^{3}	Protein mg. per 100 ml.		Cerebrospinal Fluid.	Plasma.
Msanzegwemi	M	20	53	0	0	15	Controls ; cases of relapsing fever	0	
Magalalila	M	20	49	0	2	17		0	
Masiku Nkandi	M	37	45	0	110	85	0·75	Faint trace	12·5 22 hours later
Bundala Dolo	M	25	? 50	0	80	40	0·75	Faint trace ? 0·03	7·2
Budada	M	30	80	0	30	47	95, 102, 109, 116, 150, 153	0	1·8 10 days earlier
Ndumbagwe	F	11	? 2	0	24	28	3, 10, 17, 24, 56 (¾), 70 (¾), 79 (½)	Very faint trace	17·2
Mwizalawi	F	25	32	+	560	52	0·75	Very faint trace	3·5
Masalu Manyenye*	F	30	46	0	34	22	1	Very faint trace	3·5
Juma Saindi	M	40	? 55	0	4	28 ?	1·75	Very faint trace	3·5
Malila	M	50	45	0	10	18	1·75, 9, 16, 23	Very faint trace	6·2

*This patient later relapsed while under treatment. (Details of this case will be given in a later paper.)

to this figure. Tests by a biological method were also negative. During previous work (HAWKING, 1939) it had been found that if trypanosomes of the Liverpool strain of *T. rhodesiense* were incubated in contact with Bayer 205, 0·1 mg. per ml., for three hours at 37° C. and then inoculated into mice, each mouse receiving 0·4 ml. of a suspension containing about 2,000 trypanosomes per c.mm., they failed to cause infection; a concentration of 0·01 mg. per ml. was ineffective. Accordingly, trypanosomes of the same strain were incubated at

37° C. for 20 hours in cerebrospinal fluid from the patients, *Bundala Dolo*, *Ndumbagwe* and *Mwizalawi* respectively, a small amount of serum having been added to permit survival of the trypanosomes. After 20 hours, rats were inoculated intraperitoneally, each rat receiving about 0·4 ml. of a suspension containing 30 to 120 trypanosomes per c.mm.; and in each case infection occurred. It is concluded that, although Bayer 205 was present in the plasma in considerable concentrations, it did not penetrate into the cerebrospinal fluid in sufficient amounts to be detected by the present methods. This conclusion agrees with that which has been reached by clinical experience, since Bayer 205 is seldom able completely to sterilise patients in whom the central nervous system has become involved. Yet although complete sterilisation is seldom attained in such patients, it is occasionally reached (*e.g.*, KEEVILL, 1934, describes a number of patients with changes of the cerebrospinal fluid who were cured by Bayer alone), so presumably small amounts do sometimes penetrate.

The almost insuperable difficulty with which Bayer 205 reaches the cerebrospinal fluid is in marked contrast to the ease with which tryparsamide passes in; as will be shown in later papers, the cerebrospinal fluid content of tryparsamide and its active derivatives approximate to that of the blood.

DISCUSSION.

The belief that Bayer 205 remains in the blood of animals for long periods after administration was originally reached through biological experiments: and more recently it was confirmed through the chemical estimations of DANGERFIELD, GAUNT and WORMALL (1938). The results described above show that this is true for man also. The concentrations found in human plasma are lower than those found by DANGERFIELD, GAUNT and WORMALL (1938) or by VIERTHALER and BOSELLI (1939) in the blood of rabbits and dogs, and the duration seems to be shorter. But it is difficult to compare dosages between man and the smaller animals: relatively to their weight the animals received much greater doses. The present observations have also confirmed by chemical methods the clinical conclusion that the compound does not penetrate into the cerebrospinal fluid except in minute amounts.

This knowledge of the concentration of Bayer 205 in the blood is clearly applicable in its use against sleeping sickness, although in view of the large variations observed among different individuals only rough approximations can be obtained by the use of a single standard dosage administered to all patients indiscriminately. In treatment, the ideal procedure would be rapidly to produce a high concentration in the blood during the first few days by doses in quick succession, and then to maintain this plateau for a suitable period by doses given at longer intervals. The optimum concentration for this

purpose, and the optimum period for which it should be maintained, will have to be based on further experience, controlled by chemical estimations on the blood; but consideration of Fig. 2 would suggest that it would probably be a satisfactory procedure to begin by giving doses of 1 gramme on the 1st, 2nd, and 4th days. On the 5th day, a chemical estimation of the concentration in the blood should be made and further treatment should be influenced by tl ˙ on the one hand, and upon the presence or absence of albuminuria or other toxic symptoms, on the other. In a case like that of *Mitaki* with a low concentration in the blood, more intensive treatment would be required; and conversely in a case, like *Ngaye*, with a high concentration. In average cases, further doses given on the 8th, 15th and 22nd days would possibly be suitable. In cases where a special effort is being made to raise the concentration in the blood, it should be remembered that Bayer 205 tends to accumulate; so that the aim can be reached more smoothly and safely by increasing the frequency of the injections, rather than by increasing the size of the individual doses. Moreover, BOURSNELL, DANGERFIELD and WORMALL (1939) found that a level reached in this way was longer maintained. At the end of the course, the blood should be examined again to ensure that a satisfactory concentration has indeed been obtained, since in some individuals the retention of the drug is very defective; examples of this will be described in a later paper. The definition of what should be considered as a satisfactory concentration will depend upon the accumulation of further experience. Examinations of the blood at later periods, *e.g.*, at the end of the 1st and 2nd months when possible, would also be of value for prognosis and for consideration of further treatment. Obviously these chemical estimations will be possible only in the case of Europeans or selected natives treated at a central hospital with good laboratory facilities; but since the Wormall test using a Lovibond comparator is quite simple, and the information which it yields is so valuable, its use is strongly urged in all such cases. In the outlying parts of a colonial territory, it might often be practicable to mix samples of a patient's plasma or serum with an equal volume of strong hydrochloric acid in a stoppered container, and to send it to the central laboratory for estimation. If the specimen arrives within seven or eight days, the accuracy of the determination will still be amply sufficient for all practical purposes.

In the treatment of native patients in the bush, chemical control by blood examinations will obviously be impossible, and a standard course must be designed suitable for all but very abnormal individuals. With cases treated as in-patients, there is a big advantage in giving the first two or three doses in rapid succession and in spacing the subsequent doses at longer intervals; the desired concentration in the blood is reached more rapidly, the whole period of treatment is shortened, and less harm is done if the patient absconds after the first week. During mass treatments of an anti-sleeping-sickness campaign, on the other hand, it may be considered more important to avoid confusing the

patients and native dispensers, and to keep the interval between doses always the same.

The use of Bayer 205 for prophylaxis against sleeping sickness has been the subject of much consideration, but until recent times there have been no quantitative data available as to the blood-concentrations involved. In a later paper, three patients will be described who showed a transient relapse during or shortly after treatment. At the times when trypanosomes were present in the blood, the concentration of Bayer 205 in the plasma was 4·5, 1·0 and 0·15 mg. per 100 ml. in the three cases respectively; but for reasons there discussed, it is concluded that the reappearance of the parasites in the blood of these patients is not strictly comparable with what occurs at the initiation of a new infection. However, these figures are of interest. VIERTHALER and BOSELLI (1939) found that a blood-concentration of 1·3 mg. per 100 ml. was sufficient to protect rabbits against an intravenous injection of a laboratory strain of *Trypanosoma brucei*. In the curves given above, a concentration of 0·6 mg. per 100 ml. had been reached nine days after a single injection of 1 gramme (Fig. 1), or about 180 days after a course of four injections (Fig. 4); but since there are such large individual variations, it would be unsafe to rely upon these concentrations being maintained in any particular instance. DUKE (1936) administered 1 gramme to each of nine volunteers, who were then exposed to infection either by the direct inoculation or by the bite of infective tsetse-flies; four of these volunteers were infected at the first time of testing, 73, 92, 95 and 105 days respectively after treatment, while the other five were protected for periods of 97, 120, 123, 190 and 327 days respectively. It is not possible to state what concentration would have been present in the blood at these dates, but probably it would have been too small to detect chemically. Experiments on animals will doubtless yield more information concerning the minimal effective concentration of Bayer 205 in the blood required to prevent infection, but meanwhile it may be remembered that clinical opinion is divided as to the advisability of this method of prophylaxis. While some workers recommend it, others fear that its use might produce " cryptic " infections, which, if due to *T. rhodesiense*, might be more dangerous than a frank infection diagnosed early and adequately treated. Experience alone can decide.

SUMMARY.

1. By means of the Wormall test, estimations were made on the concentration of Bayer 205 found in the plasma of African sleeping-sickness patients who had been treated with this compound. Big variations were found between one individual and another.

2. In persons who had received a single intravenous dose of 1 gramme, the average concentration was about 3 mg. per 100 ml. after two days, and about 0·6 mg. after nine days.

3. In persons who received courses of four doses, the concentrations

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reached depended more upon the individual peculiarities of the patient than upon the spacing of the doses. One day after the fourth dose, the concentration in the blood ranged from 4 to 15 mg. per 100 ml. After courses of five or six doses, Bayer 205 could be demonstrated in the blood of some patients up to eight or nine months later; in other patients it had disappeared within five months.

4. Estimations were made on the cerebrospinal fluid. Although Bayer 205 was present in the plasma of these patients in considerable concentrations, it did not penetrate into the fluid in sufficient amounts to be detected by the chemical test.

5. Since individuals vary so greatly in their tendency to retain Bayer 205 in their blood, it is strongly recommended that the treatment of European cases of sleeping-sickness be controlled by chemical estimations.

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STUDIES IN TRYPANOSOMIASIS.

IV.—NOTES ON THE SEROLOGICAL CHARACTERS OF *TRYPANOSOMA BRUCEI* AFTER CYCLICAL DEVELOPMENT IN *GLOSSINA MORSITANS*.

BY

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INTRODUCTION.

The early investigators of the serological reactions of trypanosomes were struck by the ability of these organisms to produce an apparently unending series of serological types during the course of experimental infections in animals. This aspect of biological variation has been studied by numerous observers, as for example RITZ (1916), LEUPOLD (1928) and RUSSELL (1936), who appeared chiefly interested in the number and diversity of the serological types that could be obtained. Generally speaking, these studies were carried out with old laboratory strains of trypanosomes which had been maintained, often for years, by direct passage in animals. In some cases, at least, the strains had almost certainly lost the power of cyclical development in tsetse flies and possessed a much enhanced pathogenicity for small, laboratory animals. There can be of course no question of this innate power of trypanosomes for serological variation. But some of the methods employed to evoke it (such as the exhibition of sub-curative doses of drugs) introduce a number of extraneous, uncontrolled factors. This makes it more difficult to evaluate the results obtained. Experiments of YORKE, MURGATROYD and HAWKING (1931), for example, showed that it was possible to produce drug-resistant strains of trypanosomes by the action of drugs even *in vitro*.

In the present work the problem of variation was studied from a different angle, and attempts were made to parallel as far as possible the conditions of primary infection in nature, instead of those of long-continued disease. The original strains, from which various substrains, serological types and variants of different electric charge have been derived, were three in number, *viz.* :—

(1) *Trypanosoma brucei* G.C. Isolated from a naturally infected horse in the Gold Coast into a guineapig. Second passage guineapigs received in London on 3/4/37.

(2) *T. brucei* P.T. Also isolated from a naturally infected horse in the Gold Coast into a guineapig. First passage guineapig received 28/9/38.

(3) *T. brucei* B.V. Isolated from a native dog in Uganda into a guineapig from which rats were inoculated. Second passage rats received 23/8/39.

These strains were all maintained by serial syringe-passage in rats.

In the experiments recorded below, small, laboratory animals were infected with one of the various derivatives of these recently-isolated strains. Groups·of *Glossina morsitans* (hatched in this country from pupae sent regularly from Tanganyika) were fed on these animals on a single occasion. The flies were thereafter maintained as described by BROOM (1939) and were dissected after an appropriate interval. The salivary glands of infected flies' were inoculated into young rats. Each of the " cyclical substrains " so obtained was compared serologically with the others, and also with the original strains on which the flies had fed, by means of the Red-Cell Adhesion Test (DUKE and WALLACE,1930), using the modifications suggested by BROWN and BROOM (1938).

Unfortunately it has proved impossible for the supply of glossina pupae to be continued, so the work has been stopped prematurely. Many of the experiments are therefore unfinished, but some of the results seemed to be of sufficient interest to warrant publication even in an incomplete form.

INFLUENCE OF THE SIGN OF THE CHARGE OF THE ORIGINAL STRAIN.

It has already been shown (BROOM, BROWN and HOARE, 1936) that the blood forms of trypanosomes can carry either a positive or a negative electric charge, and that reversal of the sign of the charge is associated with a relapse of infection after a temporary, spontaneous disappearance of trypanosomes from the blood stream. Pure races of electro-positive and electro-negative variants of *T. brucei* G.C. had been obtained by the inoculation of single trypanosomes into rats (BROOM and BROWN, 1939) and maintained by syringe-passage in rats. These two variants were serologically distinct and showed no cross-reactions. Batches of tsetse flies were fed on rats infected with one or other variant and were dissected 5 weeks later. Infected salivary glands were inoculated into single rats and the resulting infections were carried on by serial syringe-passage. Immune sera were obtained by administering a curative dose of Bayer 205 to the rats at the height of the infection, and bleeding them from 10 to 20 days later.

In order to compare the serological reactions of the original variants and the cyclical substrains, immune sera were prepared against four derivatives :—

(1) *T. brucei* G.C. (Pos.) : the original electro-positive variant.
(2) *T. brucei* G.C. (Neg.) : the original electro-negative variant.
(3) *T. brucei* G.C. (P₂) : a cyclical substrain isolated from a fly fed on (1) above.
(4) *T. brucei* G.C. (U₃) : a cyclical substrain isolated from a fly fed on (2) above.

Each serum was then tested against the four types of trypanosomes. The results of the experiment are given in Table I and show that the cyclical substrains do not react with the sera prepared against the original variants

TABLE I.

RED-CELL ADHESION OF ORIGINAL VARIANTS AND CYCLICAL SUBSTRAINS OF *T. brucei* G.C.

Trypanosomes.	Sera.			
	Anti-Original Positive Variant.	Anti-Original Negative Variant.	Anti-Cyclical Substrain P₃.	Anti-Cyclical Substrain U₃.
Original Positive Variant	+ + + +	O	+ +	+ +
Original Negative Variant	O	+ + + +	+ +	+ +
Cyclical Substrain P₂ ...	O	O	+ + + +	+ + + +
Cyclical Substrain U₃ ...	O	O	+ + + +	+ + + +

In this test the adhesion of individual trypanosomes to red blood corpuscles is examined microscopically.

In this and all following tables the symbols represent the percentage of trypanosomes showing adhesion, *viz.* :—

O = 0 to 10 per cent. ; ± = 10 to 20 per cent. ; + = 20 to 40 per cent.
+ + = 40 to 60 per cent. ; + + + = 60 to 80 per cent. ; + + + + = 80 to 100 per cent.

(irrespective of whether the cyclical substrain was descended from the particular variant or not) whereas each substrain does react with the immune serum of the other, in spite of the unrelated origins of the two.

It seems therefore that neither the electric charge nor even the serological type of the original infection determines the serological type of the substrain which appears after cyclical development in glossina. On the contrary, passage through tsetse flies tends to eradicate the differences between variants so that the cyclical substrains show close resemblances, if not absolute identity of serological type.

From the table it will be seen also that the immune sera of both these cyclical substrains produced red-cell adhesion with the original positively and negatively charged variants. It was suggested by Dr. A. FELIX that this probably

meant that the original variants possessed a minor antigen which was present in greater quantity in the substrains. The small amount of antigen was unable to stimulate antibody formation but it was sufficient to react with the homologous antibody in the immune sera of the cyclical substrains. Further evidence on this point will be considered later.

FURTHER EXPERIMENTS ON THE INFLUENCE OF THE ORIGINAL STRAIN.

A larger series of experiments was carried out on similar lines. Batches of tsetse flies were fed on rats infected with different serological types of *T. brucei* G.C.: these had been obtained by allowing both original and cyclical substrains to relapse spontaneously, so that a considerable variety of serological types was available. As before, immune sera were prepared both against the original types and the cyclical substrains, and each strain was tested against the sera of the others. More than fifty cyclical substrains have been examined but in no case has any one been tested against the sera of all the others. Complete cross-tests were carried out on sets of six to twelve substrains as the antisera became available, and the earlier substrains were thereafter usually discarded, to reduce the work and the animals required to maintain a large number of these serological types.

Without giving details of the many hundreds of tests carried out, it may be stated that the findings were comparable with those shown in Table I, *i.e.*, there was a close serological resemblance · among cyclical substrains, irrespective of the serological type of the infection on which the tsetse flies were fed.

If this serological similarity proved to exist among a variety of strains from different sources, and not only among the derivatives of one strain, it would obviously be of importance in the diagnosis of early cases of disease. Further strains were therefore obtained, namely *T. brucei* P.T. and *T. brucei* B.V., and similar experiments were undertaken with them. ·

It was found that, within the limits of a single strain, the substrains derived by cyclical passage through tsetse flies were all related serologically. The substrains from different original strains however were quite distinct from one another.

ABNORMAL SEROLOGICAL REACTIONS.

Although it is correct to say in general terms that the cyclical substrains of a single strain all closely resemble each other, the statement must be modified in detail because aberrant results were obtained in some instances. The discrepancies observed were of two kinds. First, where the substrain reacted poorly, if at all, with other immune sera, and its own immune serum gave little or no adhesion with other substrains; and second, cases in which the substrain and its antiserum gave the expected reactions with certain strains and abnormal ones with others.

DIFFERENCES IN INCUBATION PERIOD.

When the histories of substrains of the first type were examined carefully it was found that in certain instances a partial relapse, which had not been noticed at the time, must have occurred. The only evidence regarding the relapse might be that during a particular passage the infection did not show for 9 or 10 days instead of the usual 4 or 5 days. This was presumably due to some abnormal resistance in the rat used, and proof that the serological type can be altered in this way was shown in the following case. During the course of an experiment, two cyclical substrains of *T. brucei* P.T., A_4 and B_4, were being tested. It was found that while Substrain A_4 reacted positively with anti-A_4 and anti-B_4 sera, Substrain B_4 was negative with both. Previous results had shown the two substrains to be apparently identical, so a fresh antiserum was prepared against Substrain B_4. This immune serum produced adhesion with the B_4 substrain but not with A_4, exactly the reverse of the previous serum. Obviously the serological type of Substrain B_4 had changed since the original antiserum was prepared. These results are summarized in Table II.

TABLE II.

ADHESION OF SUBSTRAINS A_4 AND B_4 WITH TWO B_4-ANTISERA.

Trypanosomes.	Sera.		
	Anti-A_4.	Anti-B_4 (Old serum).	Anti-B_4 (New serum).
Substrain A_4	+ + + +	+ + + +	O
Substrain B_4	O	O	+ + + +

It was seen from the animal records that a recent sub-inoculation of Substrain B_4 had taken an unusual time to infect. Although no obvious relapse had occurred, it seems likely therefore that the individual rat possessed some abnormal resistance which caused the alteration of the original infection.

With this possibility in mind a review was made of previous discrepant results, particularly those in which rats, inoculated directly with infected salivary glands, showed trypanosomes of an aberrant type. Here again it had happened in some instances that a longer interval than normal elapsed between the inoculation and the first appearance of infection. It was of course impossible to prove any causal relationship in these cases, but these recently-isolated cyclical substrains are strikingly labile and liable to relapse as compared with old laboratory strains. To minimize this assumed source of error therefore, as much as possible of the transmission and passage of strains was carried out with young, laboratory-bred rats.

Discrepancies of the second type, in which the substrains gave some normal and some abnormal reactions, were more varied in character and must be dealt with singly.

DIFFERENCES IN ANTIGENIC EFFECT AND SENSITIVITY.

Early experiments with the original strain showed that a potent antiserum could be obtained from rats, 7 days after the administration of a curative dose of Bayer 205. As a routine measure, rats were not bled at a shorter interval than 10 days but sometimes they were left for longer periods.

During one series of cross-tests it was found, as shown in Table III, that Substrain X_1 reacted strongly with antisera of Substrains C_1 and R_1 but that anti-X_1 serum was without effect on any of the three substrains. Another sample of anti-X_1 serum was obtained and this produced complete adhesion of all three substrains.

TABLE III.

CROSS-ADHESION REACTIONS OF SUBSTRAINS C_1, R_1 AND X_1.

Trypanosomes.		Sera.			
		Anti-C_1.	Anti-R_1.	Anti-X_1 1st Sample.	Anti-X_1 2nd Sample.
Substrain C_1 ...	...	++++	++++	O	++++
Substrain R_1 ...	...	++++	++++	O	++++
Substrain X_1 ...	...	++++	++++	O	++++

This finding was not an isolated example but occurred with a few other strains also. The first suggested explanation, that normal serum had been mistaken for immune, had therefore to be discarded. The only difference between the two samples of sera appeared to be that the first was collected 10 days after cure of the infection, whereas the second rat had been left for 25 days after receiving the drug. The other instances in which similar results were obtained were also found to occur with sera taken at a relatively short time after administration of Bayer 205. Since all animals were treated at approximately a standard degree of infection the most likely explanation seemed to be that in these cases there was a delay in the development of antibodies, due either to lack of response by the individual rat or to poor antigenic stimulation by the particular substrain of trypanosomes.

To test this point, groups of rats were inoculated with certain substrains and were bled 7 days after the infection   cured. The antibody titres of the various sera were estimated. It was !   it rats infected with the same

substrain all showed comparable titres; on the other hand, as is shown in Table IV, there was marked variation in the antibody content of immune sera of different substrains.

TABLE IV.

VARIATION IN ANTIBODY STIMULATION BY DIFFERENT CYCLICAL SUBSTRAINS OF *T. brucei*.

Substrain	...	X_1	K_1	Q_1	J_1	C_1
Adhesion...	...	O	+	++	+++	++++

Each substrain was tested against its homologous antiserum collected 7 days after administration of Bayer 205.

FURTHER EVIDENCE ON MAJOR AND MINOR ANTIGENS.

During the course of the above experiment some of the active sera, collected at a short interval after the cure of infection, were tested against heterologous trypanosomes. Curious effects were obtained in certain cases. One example is shown in Table V in which are set forth the results of cross-adhesions of two cyclical substrains of *T. brucei* G.C., E_2 and G_2, with two samples of their antisera, collected after 7 days and 17 days respectively. In this case each 7-day serum gave good adhesion with its homologous substrain but little or none with the heterologous trypanosomes. Both 17-day sera on the other hand produced strong positive reactions with both substrains.

TABLE V.

EFFECT ON CROSS-ADHESION REACTIONS OF THE TIME INTERVAL BETWEEN CURE OF INFECTION AND COLLECTION OF SERUM.

Trypanosomes.	Sera.			
	Anti-E_2 7-Day Serum.	Anti-G_2 7-Day Serum.	Anti-E_2 17-Day Serum.	Anti-G_2 17-Day Serum.
Substrain E_2	++++	±	++++	++++
Substrain G_2	O	++++	++++	++++

By analogy with serological findings in bacteriology, two possible explanations suggested themselves :—

(1) That these substrains consisted of individuals of different serological characteristics, some in the " specific phase " and some in the " group phase "

—to employ bacteriological nomenclature. The time at which " specific ", and " group " antibodies appeared would then depend on the relative proportions of the two phases in the particular substrain.

(2) That each trypanosome possessed a type-specific antigen and also a common or group antigen. The specific antigen elicited an earlier antibody response either because it was quantitatively the major antigen or else because of a qualitative difference between the two.

An attempt was made to test the first suggestion by the same sort of method as was used by ANDREWES (1922) in the case of bacteria. Rats were infected with single trypanosomes and the resulting infections tested. In no instance was there any indication of a separation into specific and group phases. On the contrary all infections showed the double serological characters of the parent strain.

TABLE VI.

CROSS-ADHESION OF SUBSTRAINS Q_1 AND R_1 WITH ANTISERA PREPARED ON DIFFERENT DATES.

Trypanosomes.	Sera.			
	Anti-Q_1 of 22/9/38.	Anti-R_1 of 23/9/38.	Anti-Q_1 of 24/1/39.	Anti-R_1 of 15/1/39.
Substrain Q_1 (19/4/39)	$+ + + +$ (1/16)	$+ + + +$ (1/32)	$+ + + +$ (1/16)	O
Substrain R_1 (19/4/39)	$+ + + +$ (1/8)	$+ + + +$ (1/16)	O	$+ + + +$ (1/16)

The fractions indicate the highest dilution of serum giving complete adhesion.
O = no adhesion in a dilution of one in two.

As regards the possibility of the presence of specific and group antigens, further evidence was obtained from the examination of two other cyclical substrains, Q_1 and R_1. These were isolated from flies which had fed respectively on the electro-positive and electro-negative variants of *T. brucei* G.C. The original serological tests seemed to show that these two substrains were closely related : each gave adhesion to approximately the same titre with both immune sera, but no absorption tests were done at that time. After syringe-passage in rats for some six months these substrains were used in further experiments. It was then found that the antisera collected shortly after the substrains were isolated still gave good adhesion with both substrains, but that recently-prepared immune sera were strictly specific. The results of this test are given in Table VI, in which the titres of the sera are shown in brackets. (It will be noted that Substrain Q_1 is a more sensitive indicator than Substrain R_1, since Q_1 gives a higher titre with both its homologous and heterologous antisera.)

A series of antibody-absorption experiments was then performed. The four sera, diluted one in two with physiological saline, were repeatedly absorbed with large numbers of each substrain and the residual titres determined. The results are set out in Table VII : in each section of the table the first line gives the titre of the serum before absorption, the second line the titre after absorption with the homologous substrain, and the third line the titre after absorption with the heterologous trypanosomes.

TABLE VII.

THE ABSORPTION OF FOUR ANTISERA WITH SUBSTRAINS Q_1 AND R_1.

Sera.	Titre against Substrain Q_1.	Titre against Substrain R_1.
Anti-Q_1 of 22/9/38		
(1) Unabsorbed	1/16	1/8
(2) Absorbed with Substrain Q_1	0	0
(3) ,, ,, ,, R_1	1/8	0
Anti-Q_1 of 24/1/39		
(1) Unabsorbed	1/16	0
(2) Absorbed with Substrain Q_1	0	0
(3) ,, ,, ,, R_1	1/16	0
Anti-R_1 of 23/9/38		
(1) Unabsorbed	1/32	1/16
(2) Absorbed with Substrain R_1	1/2	0
(3) ,, ,, ,, Q_1	1/8	1/4
Anti-R_1 of 15/1/39		
(1) Unabsorbed	0	1/16
(2) Absorbed with Substrain R_1	0	0
(3) ,, ,, ,, Q_1	0	1/16

The symbols have the same meaning as in Table VI.

It is apparent that, in all cases save one, the homologous substrain removed the antibodies for both substrains from the old and the recently-prepared sera. The exception (R_1 antiserum of 23/9/38, absorbed with Substrain R_1 and tested against Substrain Q_1) is of little importance since the titre was reduced from 1/32 to 1/2, and it has been already pointed out that Substrain Q_1 is a very sensitive indicator. In addition, although the heterologous substrains had no effect on the recent sera, they reduced the titres of the old sera for the homologous trypanosomes.

Despite the relatively low titres of the sera it proved impossible to remove all the antibodies, but this may have been due, in part at least, to technical

difficulties. It was found that frequent incubation of serum with the very heavy suspensions of trypanosomes necessary for absorption tended to produce a gel-like condition of the mixture from which little or no fluid could be centrifuged off. One had therefore to stop short of this point even at the cost of incomplete absorption.

DISCUSSION.

Before discussing these results it might be well to summarize the findings :—

(1) When first isolated from tsetse flies, two cyclical substrains, Q_1 and R_1, reacted strongly with the antiserum prepared against either substrain.

(2) Six months later no cross-reactions were obtained with recently-prepared immune sera, *i.e.*, against the substrains as they then were.

(3) But each substrain still gave good red-cell adhesion with both the original antisera, heterologous as well as homologous.

The first two facts could be explained quite easily by analogy with bacteria. It is necessary to assume only that each substrain contains two antigens, one specific to itself and the second common to both. Continued syringe-passage resulted in the loss of the common or group antigen, leaving only the specific.[*] The loss of an antigen could be compared to the disappearance of the capsule of Friedländer's bacillus or the suppression of H antigen in the salmonella group of bacteria. Certainly these alterations are brought about during growth on artificial media, but one might argue that syringe-passage is an unnatural method for the propagation of the brucei group of trypanosomes and might also lead to some deterioration of the organisms. It must be remembered, however, that the loss is not complete. Although no " group " antibodies are formed, the trypanosomes still react with the antibodies present in the old, heterologous sera.

Reference back to Table I will show that a somewhat similar, but reversed, finding was recorded there. The original variants of different electric charge were themselves serologically distinct, but each reacted with both the antisera prepared against the cyclical substrains, P_3 and U_3, derived from them.

Disregarding for the moment the differences between these two examples, it may be said that in each case there are two types, apparently serologically distinct, which both react with a single serum. With regard to the first instance it was suggested that the result could be explained by supposing that the original variants possessed a common, minor antigen which was insufficient in quantity to promote antibody formation. The cyclical substrains had the same antibody in greater amount and produced antibodies with which the original variants were able to react. If that be so, it could be assumed in the case of Substrains

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[*]This change was not due to a relapse so far as could be determined, and since the two substrains acted in exactly the same way it seems unlikely that relapses could have taken place, unnoticed, in both cases.

Q_1 and R_1, that they had developed the group antigen (during cyclical development in glossina) to an extent sufficient to stimulate antibody formation. In course of time however the antigen gradually became reduced in amount until it was no longer effective.

Another possibility is to look on these reacting, but non-antigenic, groups as being similar to haptens which have the same characteristics. Haptens react with antibodies, although they are unable to stimulate their formation except when attached to a protein molecule : in that case the hapten, and not the protein, determines the specificity of the antigen. If, then, it could be imagined that the configuration of the molecule became altered in some way so that the hapten was no longer effective as an antigen, a condition such as was actually found might well arise. Although haptens are more commonly carbohydrates, some are of a lipoid nature, e.g., the Forssman antigen (TANIGUCHI, 1921). KLIGLER and OLITZKI (1936) analyzed T. evansi and found that these trypanosomes consisted of nearly 60 per cent. lipoids and only 25 per cent. protein compared with 12 to 15 per cent. lipoids and 44 per cent. protein in the case of Bacterium shigae. One might assume therefore that only a relatively small proportion of the lipoids could be combined with the protein of trypanosomes. If the combination is labile, and the ease with which serological variation is induced suggests that it must be so, then conditions might occur in which rearrangement of the lipoid-protein complex took place. As a result the antigenic constitution and hence the serological individuality of the strain would be altered, but the capacity of the hapten to react with its homologous antibody would not necessarily be lost.

EXPERIMENTS ON GROWTH IN DEVELOPING EGG.

The use of developing hen's eggs is mainly confined to the study of filter-passing viruses but it has been possible to employ eggs as a culture medium for certain protozoa. BIOCCA (1938), LONGLEY, CLAUSEN and TATUM (1939) and MITCHELL, WALKER, HEATH and McKERCHER (1939) have successfully maintained trypanosomes in this way.

A few experiments were carried out to test the effect on serological reaction of the growth of trypanosomes in the developing egg. The technique used was that described by BURNET (1936). In these experiments eggs, incubated for 10 to 12 days, were inoculated with substrains of T. brucei G.C. One week later, washings of the chorio-allantoic membrane were injected into young rats and the reactions of the resultant infections tested. It proved easy to transmit infection in this manner in most cases. The embryos in a certain number of the eggs died a few days after inoculation, but no trypanosomes could be recovered from them. It cannot be said therefore whether the infection had proved lethal or the embryos had been damaged by the manipulations necessary to introduce the trypanosomes.

These " egg-substrains " were examined in the same way as the cyclical substrains. As however they varied serologically from the strains inoculated, and also from each other, the question was not pursued further. Obviously, growth in developing eggs provides another method of inducing serological variation, but that was not within the scope of the present study.

SUMMARY.

1. Experiments were undertaken to study the effect of cyclical development in *Glossina morsitans* on the serological characters of *Trypanosoma brucei*.

2. In general, the " cyclical substrains " which result from this method of passage resemble each other closely irrespective of the serological type of the strain on which the flies were fed.

3. This is true only of the derivatives of a single original strain. The cyclical substrains of different original strains are serologically distinct.

4. In spite of the general similarity within the limits of a single strain, there are certain differences in detailed serological reactions. Examples of these are given and their import discussed.

5. A strain of *T. brucei* was successfully cultivated in developing hens' eggs. The serological type of the trypanosomes was changed in an irregular manner during the process.

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ONCHOCERCA VOLVULUS AND ITS VECTOR IN THE SOUTH KAVIRONDO DISTRICT OF KENYA.

BY

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INTRODUCTION.

The discovery of *Onchocerca volvulus* by Dr. G. L. TIMMS, Pathologist at the Medical Research Laboratory, Nairobi, in a nodule submitted from Kakamega (in the North Kavirondo district of Nyanza Province) in June, 1938, and the resulting preliminary sampling of the people by Dr. F. HAWKING (1939) in Kakamega, were followed by a discovery by Dr. G. B. HARRIS of a focus of long standing infection in the native reserve about 20 miles north-west of Kisii in the South Kavirondo district.

Data here presented were collected during a preliminary investigation of this area and its people.

E

. *Description of Area.*

The area concerned lies roughly between 0°29′ and 0°38′ south latitude and 34°44′ and 34°38′ east longitude, on the western edge of the Kisii highlands about 12 miles south of Kendu (on Lake Victoria). The altitude varies between 4,000 and 5,000 feet. (See Map I.)

The rivers Kitare and Sanda, rising in the Kisii highlands to the east, enter this area on its eastern border and flow in a north-westerly direction to a point south of Kodera where they join and continue as the River Awach to the Kavirondo Gulf. The Kitare, carrying a bigger volume of water, is the more important of the two. It drops by a series of small waterfalls and cascades, about 1,000 feet, between the Kisii-Kendu Road and Kodera, a distance of about 10 miles. Most of the falls, however, are confined to about 1½ miles between Tributaries 1 and 2, where the river runs through a deep chasm, with steep banks densely covered with trees and bush. Cultivation is not attempted within about a mile of the river on each side. (Plates I and II).

The Sanda River enters the area about four miles to the north of the Kitare at a lower altitude. It has fewer waterfalls and cascades. Its banks are not so steep and are but sparsely covered with bush, and open areas of cultivation occur along them.

About 4 miles north of Kodera the Awach enters a vast, open plain, broken only by small abruptly rocky hills. In the next 10 miles of its course to the lake it drops only about 150 feet.

Climate.

This part of South Kavirondo enjoys an equable climate—hot though not excessively so, and not unduly humid, with an average rainfall of about 60 inches a year.

People.

Some four-fifths of the total area, which has a population of about 4,000, is occupied by people of the Luo tribe (Nilotic) and the remainder by Kisii (Bantu). Both tribes are agriculturists and do not differ much, if at all, in their domestic habits or general activities.

Their contact with European administration and culture is indicated more obviously in the clothes they wear and in the number of bicycles they own than in their methods of agriculture and husbandry, though undoubtedly there has been much progress in crop production and improvement in general public health since British administration began.

Activities.—The people live in small round huts, grass thatched, with mud walls, built in clusters of four or five called a *boma*. The huts are surrounded

by a euphorbia hedge planted to serve as a wind brake and probably also to provide a certain amount of privacy.   Cattle and goats are allowed to graze throughout the day, usually under the charge of small boys, but are driven into the *boma* for safety at night.   The rough work of tilling and breaking the soil is undertaken by adult male members of the family with the help of the females,

MAP I.
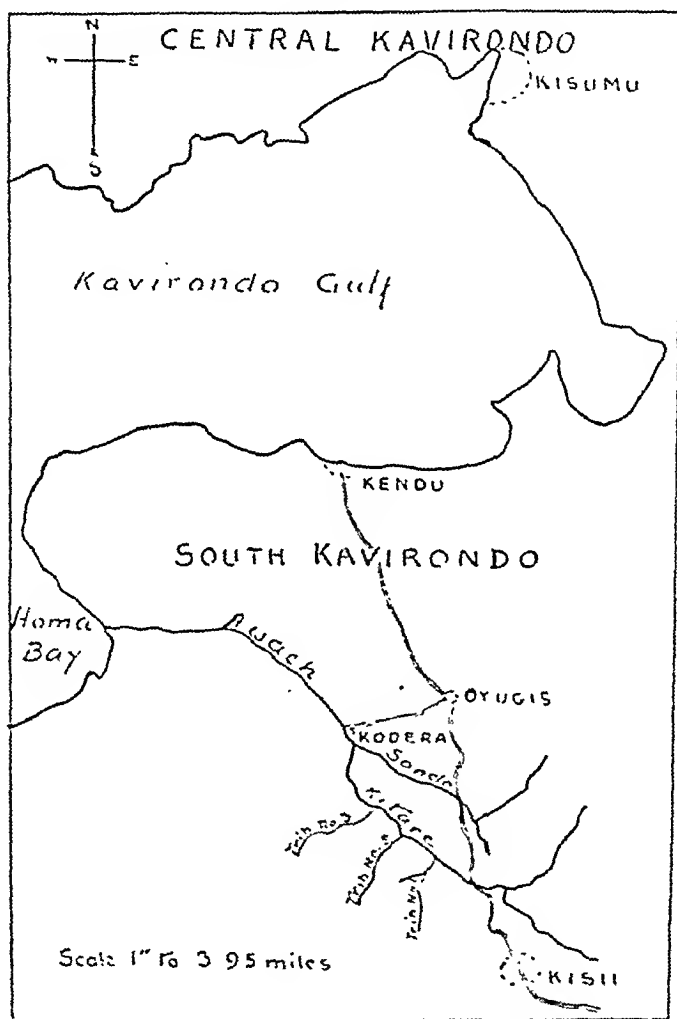
but attention to growing crops, weeding and reaping is usually regarded as the exclusive duty of the women folk.   Women also collect the fuel and water for domestic use.   Men pursue other activities such as housebuilding and trading and sometimes cattle herding.   Boys begin herding at the very early age of 4 or 5 and girls aged about 7 are usually employed tending their smaller brothers and sisters while their mothers are working on the land or attending market.

The daily vocations of the people constantly bring them into close contact with the rivers. Those who live in close proximity to rivers rely on them for their domestic water supply and collect at least a part of their fuel from the densely wooded banks. The men roam the wooded banks in search of long straight poles for housebuilding, boys graze their cattle near the rivers where there is shade, convenient water and the best of grass. Those people who reside to the west of Kitare make frequent visits to Kodera and Oyugis markets for the purposes of trade. These excursions necessitate the fording of one or other of the rivers, during which they will always pause to wash or cool themselves or to rest or chat in the shade before continuing their journey. River crossings are frequently the meeting places of herds of cattle and considerable numbers of people.

TABLE A.   (See Map II.)

INCIDENCE OF ONCHOCERCIASIS IN KODERA AREA.

Location.	No. of people examined.	No. of people infected.	Percentage infected.
Haroun	60	23	38
Obudu	62	20	32
Okudu	76	10	13
Odede A	60	27	45
Odede B	47	35	74
Josia A	60	44	73
Josia B	60	59	98
Josia C	60	47	78
Omoi A	60	23	38
Omoi B	60	23	38
Totals	605	311	51 per cent.

The area is well watered and fertile and, except for the lands adjoining the infested bush, supports a dense population. Crops produced include wimbi (*Eleusine corracana*), mtama (*Sorghum vulgare*), maize, ground nuts, cassava or manioc, sweet potatoes and sugar as well as tobacco for local consumption and cotton for export. Cattle and goats are grazed in fairly large numbers.

Agricultural produce is sold at Kodera and Oyugis. The Kodera market is held every Wednesday and such foods as ground nuts and bananas as well as tobacco are sold for local consumption. A bigger market is held every Friday at Oyugis where livestock as well as surplus farm produce are bartered or sold. Indian traders buy large quantities of grain for export whilst all the cotton is purchased by the agents of a local European ginning firm.

INFECTION IN PEOPLE.

This inquiry formed part of a more complete examination of samples of the population made in co-operation with Dr. B. P. HARRIS. His data on the medical and clinical aspects are being presented separately. These notes deal

MAP II.

Incidence of onchocerciasis Kodera Area.
Percentage infection in people.

only with the endemicity of the disease as determined by the presence of filariae in the skin.

The sampling was carried out in five centres in order to obtain as far as possible an idea of the extent and intensity of infection.

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(a) TECHNIQUE.

Each person was injected subcutaneously in the left forearm with a 1 per cent. solution of planocaine and a section of skin measuring roughly 5 × 3 mm. was removed and put in a Widal tube containing 2 to 3 drops of normal saline. After standing for a period of 2 hours the skin snip was discarded and a coverslip preparation was made with the saline. A search was then made for microfilariae with the aid of a two-thirds objective.

Six hundred and five people (men, women and children) were examined; 309 (51 per cent.) were positive for *O. volvulus* (see Table A and Map I). A daily average of sixty skin samples was obtained with the help of four African assistants.

(b) DISTRIBUTION OF INFECTION.

Only one skin snip was taken from each person. Re-examination of those who were negative would probably have increased the positives very considerably.

The main focus was found to exist along the Kitare from Tributary No. 1 to a point north of the junction of Tributary No. 3. In this area the infection rate was 98 per cent. It was expected that the percentage infection in people who inhabit areas at the heads of the tributaries would show a sharp decline but this did not prove to be the case. According to subsequent information these people at one time lived along the Kitare but had moved because of the fear of contracting the disease. Higher up the Kitare, towards the main road, the rate of infection was only 38 per cent. This sudden drop is related to the equally sharp decrease in fly density (see Table E and Map III).

The highest infection rates were found in people living on both sides of the middle portion of the Kitare river and the lowest (13 per cent.), in an open area well removed from both Sanda and Kitare rivers. (Map III and Plate II).

(c) INCIDENCE IN TRIBES AND AGE GROUPS.

Of the 605 examinations made 138 have been omitted from this analysis (Table B) because, owing to a clerical error in the first day's work, one of the people examined was not recorded. The point at which the omission occurred was not discovered, so that an unknown number of people examined were given identification numbers which belonged to previous cases.

The mean of the two adult rates—60 per cent.—is, as one might expect, higher than the mean of the two children's rates—43 per cent. It is interesting that the male rates are higher than the female in both main groups, 47 per cent. as compared with 39 per cent. in Group I and 67 per cent. as compared with 53 per cent. in Group II.

Infection rates in Luo male and female adults were 86 per cent. and 51 per cent. respectively; and in Kisii 29 per cent. and 59 per cent. This anomaly, if

it really exists, cannot be explained at the moment though it may be pointed out that the Kisii people live in the highest parts of the area surveyed and some distance away from what appear to be the most heavily infested districts (see Maps II and III).

Infection in children of the two tribes cannot be compared because of the small number examined.

TABLE B.

Tribe.	Number examined.	GROUP I. Children 0 to 16 years. Males.		Females.		GROUP II. Adults (at least 17 years). Males.		Females.	
		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Luo									
Obudu	36	—	1	—	—	8	7	6	14
Okudu	26	—	3	—	1	1	10	1	10
Odede A	60	4	13	2	8	19	8	2	4
Odede B	47	9	1	3	3	18	3	5	5
Josia A	60	4	5	3	2	25	3	13	5
Josia B	60	5	—	3	—	36	—	15	1
Josia C	60	6	1	2	3	32	2	7	7.
Kisii									
Omoi A	60	3	5	2	6	11	23	6	4
Omoi B	59	—	5	—	—	8	22	14	10
Totals		31	34	15	23	158	78	69	60
TOTAL-INFECTION RATE expressed as per cent. (both sexes) ...		47 per cent.		39 per cent.		67 per cent.		53 per cent.	

(d) INFECTION IN AGED PEOPLE OVER 60 AND CHILDREN BELOW THE AGE OF 6 YEARS.

The data in Table D are submitted merely to show that children may become infected at the age of about 4 years or earlier. As regards the aged non-infected people shown in Table C, it will be noted that they occurred in samples taken from areas showing less than 40 per cent. infection, and that where the infection rate was higher than this all old people were infected.

The ages of old people were estimated on their decrepit appearance and are therefore approximate only.

Unfortunately children in arms were not obtainable. In all twenty-two children between the ages of 4 and 6 years were examined. Of these seven were positive—an infection rate of 32 per cent. (See Table D.)

TABLE C.

INFECTION IN OLD PEOPLE.

Tribe and location.	Infection rate for area.	Number over 60 years examined.	Males.		Females.	
			Pos.	Neg.	Pos.	Neg.
LUO :						
Obudu	32 per cent.	7	3	1	1	2
Okudu	13 ,,	8	1	3	—	4
Odede A	45 ,,	6	6	—	—	—
Odede B	74 ,,	Nil	—	—	—	—
Josia A	73 ,,	1	1	—	—	—
Josia B	98 ,,	2	2	—	—	—
Josia C	78 ,,	5	5	—	—	—
KISII :						
Omoi A	38 ,,	10	1	2	4	1
Omoi B	38 ,,	14	2	5	3	4
	Totals	51	21	11	8	11

TABLE D.

INFECTION IN CHILDREN.

Tribe and location.	Infection rate for area.	Number of children below the age of 6 years examined.	Positive.	Negative.
LUO :				
Okudu	13 per cent.	2	—	2
Odede A	45 ,,	9	2	7
Odede B	74 ,,	2	1	1
Josia A	73 ,,	6	3	3
KISII :				
Omoi A	38 ,,	3	1	2
	Totals	22	7	15

THE VECTOR.

The search for the vector included intensive collecting of adult *Simulium* in bush along and near the rivers and of pupae in all parts of the rivers and their tributaries.

(a) ADULT SURVEY.

Two African assistants were employed continuously on this work with the help of various individuals of the local population. Since hand-nets proved to be of no value the searchers were provided with suction tubes and with these

MAP III.
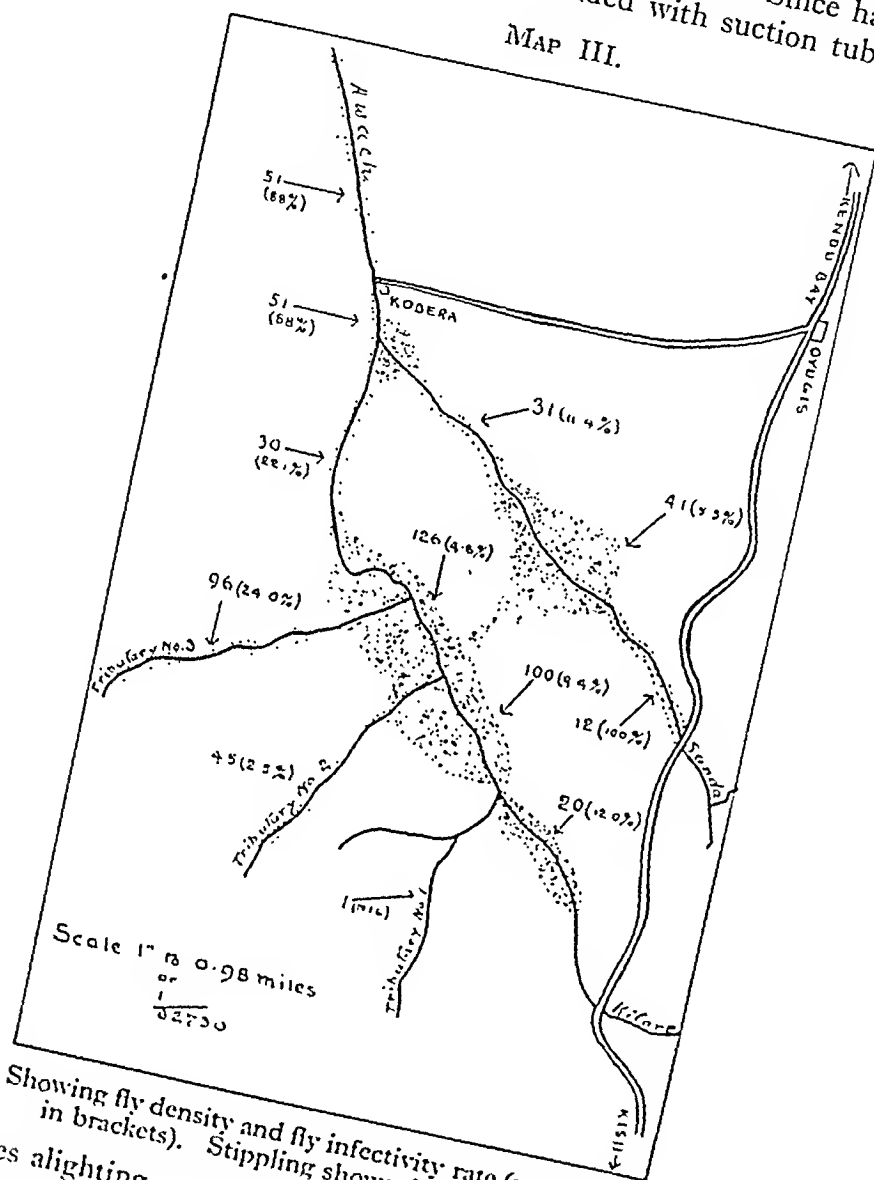
Showing fly density and fly infectivity rate (percentage in brackets). Stippling shows degree of bush: they captured flies alighting on themselves and on people working in the fields or visiting the rivers.

A total of 1,369 females was caught. With the exception of one specimen of *S. dentulosum* Roubaud, all were *S. neavei*. The one *S. dentulosum* was caught on a boy where apparently it proposed to feed. No male specimens were caught.

(i) *Fly Index* (*see Map. III, Table E and Plate II*).

Greatest adult density occurred between Tributaries 2 and 3 of the Kitare River with an index of 126. This part of the Kitare is densely wooded on both banks and contains a number of waterfalls and cascades but not nearly so many as that portion between Tributaries 1 and 2 which shows a lower density. It might well be that fly production is greater in the more rocky portions but that adults migrate to the lower regions and linger there for feeding purposes. A second catch was made later on these two portions of the Kitare on the same day with similar results.

TABLE E.

FLY INDEX (FROM ADULT CATCHES).

SUMMARY OF ALL CATCHES.

Source (Rivers and Tributaries).	Average number of boys engaged per hour.	Total number of hours of catches.	Number of flies caught. Total.	Fly index.
Awach	2·6	21·5	132	51
Kitare (between road and Tributary No. 1) ...	5·5	15·5	72	20
Kitare (between Tributaries Nos. 1 and 2)	2	47·5	399	100
Kitare (between Tributaries Nos. 2 and 3)	2·4	34·5	433	126
Kitare (between Tributary No. 3 and Awach) ...	2·5	9	29	30
Tributary No. 1	6	8	2	1
Tributary No. 2	3	14·5	82	45
Tributary No. 3	2	3	24	96
Sanda (Upper)	1	4	2	12
Sanda (Middle)	3	11	56	40·7
Sanda (Lower)	3	11·5	45	31

It is seen that density of fly varied with density of bush—at least to a great extent. The lowest indices were recorded on the upper parts of the two main rivers where bush gave place to grass and tall reeds and on a tributary of the Kitare with no bordering vegetation at all. Adult distribution is probably dependent upon shade and resulting humidity, though food supplies must of course constitute an attraction.

Table E gives the "fly index" for the localities: this is the number of flies one boy would catch in 24 hours, and is based on the work of several boys, for various periods.

(ii) Percentage of Flies Infected (Table F and Map III).

Five hundred and fifty-seven flies were dissected; seventy-three of these are not included in the analysis as they were preserved in spirit and were not dissected until after a period of 5 weeks had elapsed. In these latter specimens it is possible that some filariae became embedded in the flies' tissue and during the process of dissection were destroyed. The flies tabulated in Table F were dissected on the day on which they were caught.

No attempt was made to ascertain the seat of infection: *i.e.* proboscis, thorax, or stomach; the fly was dissected, mounted as a unit and recorded as

TABLE F.

INFECTIVITY RATES OF *S. neavei.*

Source (Rivers and Tributaries).	Number of flies dissected.	Number of flies positive.	Percentage infected.
Awach	57	5	8·8
Kitare (between road and Tributary No. 1) ...	50	6	12·0
Kitare (between Tributaries Nos. 1 and 2)	160	15	9·4
Kitare (between Tributaries Nos. 2 and 3)	42	2	4·8
Kitare (between Tributaries No. 3 and Awach) ...	22	5	22·7
Tributary No. 1	2	Nil	Nil
Tributary No. 2	40	1	2·5
Tributary No. 3	25	6	24·0
Sanda (Upper)	2	2	100
Sanda (Middle)	36	2	5·5
Sanda (Lower)	44	5	11·4

either positive or negative. All stages of the larvae as described by BLACKLOCK (1926) were seen.

Highest infectivity rates were found in lowest fly densities. The fly is reluctant, apparently, to leave dense riverine bush but when it does migrate to comparatively open country, it is in much more close contact with the human population and consequently more liable to become infected. This is emphasised by the catch made in the upper reaches of the Sanda where only two flies were caught—both were infected with O. volvulus.

It was noticed during the course of routine dissections that the highest percentage of infected flies was in catches made near fords and other places where people tend to congregate. No special effort was made to obtain specific data on this point.

(i) *Fly Index (see Map. III, Table E and Plate II).*

Greatest adult density occurred between Tributaries 2 and 3 of the Kitare River with an index of 126. This part of the Kitare is densely wooded on both banks and contains a number of waterfalls and cascades but not nearly so many as that portion between Tributaries 1 and 2 which shows a lower density. It might well be that fly production is greater in the more rocky portions but that adults migrate to the lower regions and linger there for feeding purposes. A second catch was made later on these two portions of the Kitare on the same day with similar results.

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Sanda (Upper)	1	4	2	12
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It is seen that density of fly varied with density of bush—at least to a great extent. The lowest indices were recorded on the upper parts of the two main rivers where bush gave place to grass and tall reeds and on a tributary of the Kitare with no bordering vegetation at all. Adult distribution is probably dependent upon shade and resulting humidity, though food supplies must of course constitute an attraction.

Table E gives the " fly index " for the localities : this is the number of flies one boy would catch in 24 hours, and is based on the work of several boys, for various periods.

The region of lowest infection (13 per cent.) was Okudu's location which is at its nearest point 1½ miles east of the Sanda. The people have little cause to frequent the river as they draw their domestic water supply from a small sunlit stream which runs through their land and tend their cattle and goats in their own location which is for the most part free from bush and consequently difficult country for the fly to exist in. Pole cutting excursions and visits to Kodera market probably account for most of the infection in this area.

(iv) *Activities on Hot as compared with Cold Days.*

Extracts from records of routine catches are presented in Table G, to indicate that *S. neavei* are active on hot sunny days. Temperatures were not measured.

TABLE H.

Time.	Dense shade on river bank.	Light shade on river bank.	Light shade 100 yards from river.	Light shade 200 yards from river.
8 a.m.	—	—	2	—
8.30 a.m.	—	—	2	4
9 a.m.	—	2	—	1
9.30 a.m.	—	6	—	—
10 a.m.	—	—	2	—
10.30 a.m.	—	—	1	1
11 a.m.	—	—	2	—
11.30 a.m.	1	1	—	—
12 noon	—	1	—	1
12.30 p.m.	—	1	1	—
1 p.m.	2*	3	—	—

* These were caught in light shade—no dense thicket hereabouts.

(v) *Range of Flight from River.*

Experimental catches were organized with the intention of obtaining some indication of the influence of shade and weather conditions on range of flight.

Tables H, J, K and L cannot be compared one with another because the catches were made under a variety of conditions.

Table H.—These catches were made on the east bank of the Sanda River on a dull day. Four searchers were employed. No. 1 was stationed in dense thicket on the river bank, No. 2 in light shade on the bank about 10 yards from No. 1, No. 3 in light shade 100 yards back and away and No. 4 in light shade 200 yards away from the river. Each catch was made in about 10 minutes and after each catch the searchers moved along stream about 600 to 800 yards.

(iii) *Fly Density and Disease.*

There is apparently a close correlation between fly-density and the incidence of onchocerciasis in people. In regions of high fly density a high incidence of onchocerciasis was found to exist and in regions of low fly density a corresponding drop in the degree of infection is evident (see Maps II and III).

In the middle reaches of the Kitare in regions of high fly density (100 and 126) 98 per cent. of the population was found to be infected. In the upper

TABLE G.

HOT DAYS.

Number of flies caught.	Number of searchers engaged.	Number of flies per boy hour.	Where caught. (Rivers and Tributaries)
13	2	1·8	Kitare
31	2	4·4	Kitare
4	3	0·4	Sanda
54	2	7·7	Kitare
18	2	2·6	Sanda
72	2	10·3	Kitare
23	3	2·2	Sanda
27	3	2·6	Awach
22	3	2·1	Kitare
12	3	1·1	Kitare
COLD DAYS.			
61	2	8·7	Kitare
21	2	3·0	Tributary No. 2
15	2	2·1	Tributary No. 2
34	4	2·4	Sanda
39	2	5·6	Tributary No. 3
101	3	9·6	Kitare

NOTE.—The average duration of the daily search for adults was $3\frac{1}{2}$ hours.

reaches of the Kitare near the main road in a region of low fly density (20) only 38 per cent. of the people were infected. In the vicinity of the tributaries which have fly densities of 1, 45 and 96, the human population was found to be infected to the extent of 78 per cent. This apparent inconsistency is explained by the fact that some of these people lived at one time along the Kitare and that most of them make frequent trips to Kodera and Oyugis markets.

The population on the eastern banks of the Sanda was found to have an average infection of 35 per cent.: this is consistent with the low fly densities of 12, 41 and 31.

Cattle and people at a ford in the Sanda River.
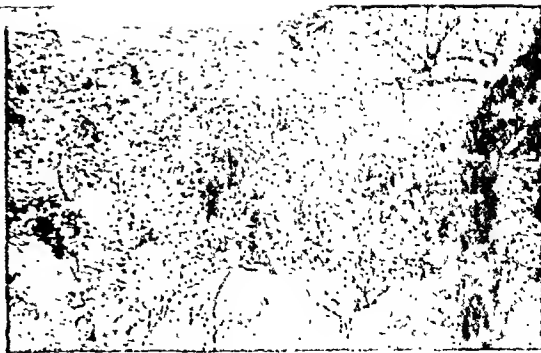
Dense bush and steep banks of the Kitare River. A section of the river is visible just beyond the African standing in the foreground.

Cascades on the Kitare favoured by *S. lepidum*.

Type of light bush in which *S. neavei* was found 700 yards from the river.
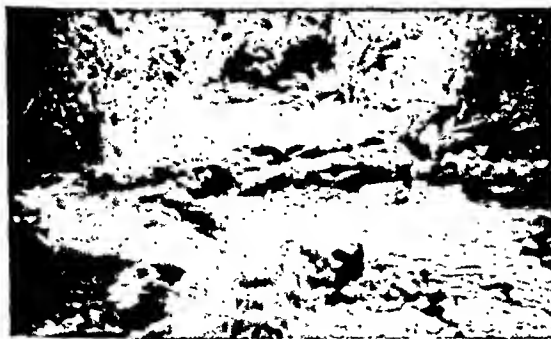
Small rocky waterfall on the Kitare, favoured by *S. lepidum* particularly.
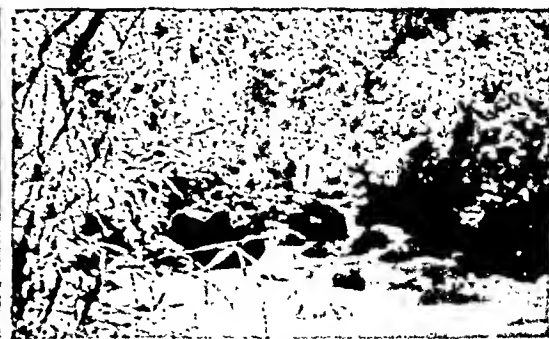
The Kitare River in the dry season (Nov., 1939). Long overhanging grass provides anchorage for *S. hirsutum* when the river rises to submerge the tips. The rocks, when covered, produce several species but particularly *S. lepidum*.

PLATE I.

Dense thicket is apparently not attractive, perhaps because of its physical hindrances to flight.

Table J.—The technique was similar to that described for Table H. A more lightly wooded part of the east bank of the Sanda in a fly density of 31 was selected for this catch and this may account for the fewer flies.

TABLE J.

Time.	Light shade on river bank.	Light shade 100 yards from river.	Light shade 200 yards from river.	Light shade 300 yards from river.
8.30 a.m.	—	—	1	—
9 a.m.	2	1	4	—
9.30 a.m.	—	3	3	—
10 a.m.	—	—	—	—
10.30 a.m.	—	—	—	—
11 a.m.	—	—	—	—
11.30 a.m.	1	—	—	—
12 noon	—	—	—	—
12.30 p.m.	2	—	—	—
1 p.m.	—	2	1	1

The first 200 yards of river vegetation would appear to harbour the biggest proportion of adults.

Catches shown in Tables H and J were carried out in densities of 41 and 31 (see Map III).

TABLE K.

Time.	Light shade 100 yards from river.	Light shade 200 yards from river.	Light shade 300 yards from river.
9 a.m.	13	21	3
9.30 a.m.	11	5	2
10 a.m.	3	8	7
10.30 a.m.	2	3	2
11 a.m.	2	2	2
11.30 a.m.	4	5	2
12 noon	—	2	—
12.30 p.m.	2	—	—

Table K.—These observations were made on the east bank of the Kitare, in a density of 100 on a dull day. Technique was as already described but with three searchers.

The unusually large catch at 9 a.m. is probably due to the fact that the searchers were in position about 30 minutes before catching commenced. It suggests that flics are attracted to a possible host or that they make food hunting patrols, or both.

It appears that on a dull day adults travel quite readily through light bush for a distance of 300 yards from their breeding grounds.

Table L.—Catches were made with three searchers in the area of highest density on the east bank of the Kitare River on a hot bright day in light bush.

TABLE L.

Catching points.	400 yards from river.	500 yards from river.	600-700 yards from river.
1st	—	—	—
2nd	2*	1*	—
3rd	2*	3*	1*
4th	—*	—	—
5th	—*	—	—
6th	1*	—	1*
7th	1*	—*	—*

Catching points, marked thus *, indicate the presence of shrubs and trees affording shade
It is noteworthy that flies seem reluctant to attack in open glades at any distance from river.

It would appear that 700 yards is approaching the maximum range of flight, though the range may be extended during dull weather.

(vi) *Influence of Shade.*

Table M gives the results of catches made simultaneously on sunny days by two assistants, one stationed in shade and the other about 50 yards from the

TABLE M.

	Shade.	Open glades.
1st catch	12	3
2nd „	3	—
3rd „	7	—
4th „	1	—
5th „	19	5

nearest shade of any kind. Shade as one would expect appears to be made use of by the majority of adults.
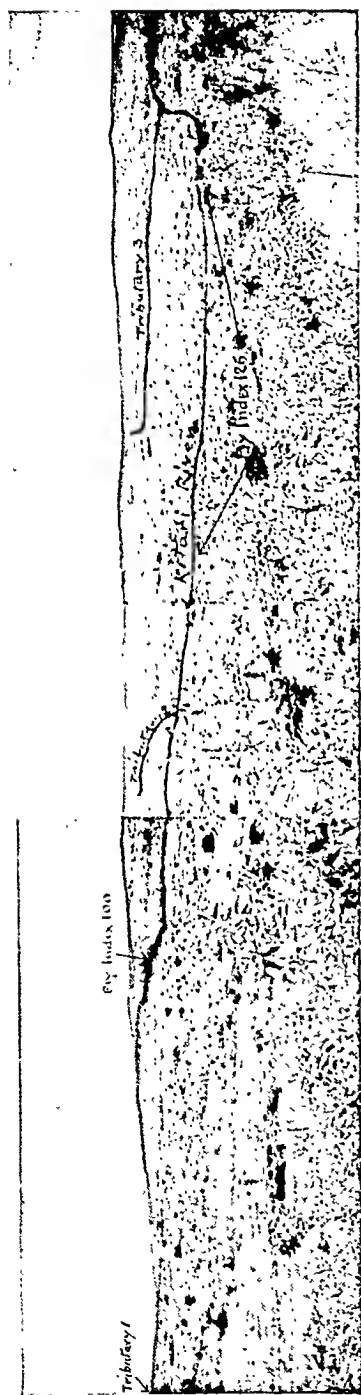
Kitare River showing fly density.

Sanda River showing bush and fly density.

PLATE II.

Two hundred and fifty-three female *S. neavei* were released in the cage containing the rabbit and the guineapig and 241 female *S. neavei* were released in the rats' cage. Inspections were made at frequent intervals. The flies were apparently reluctant to feed as they were always seen to be resting on the sides of the cages. The same reluctance was exhibited towards the monkey. Flies were applied to the monkey's back on three successive days but were not observed to bite in one single instance.

All animals are however still under observation.

(x) Contact between *S. neavei* and Population.

Contact between people and fly begins early and probably continues throughout life. Boys begin to herd cattle and goats along the river valleys about the age of 4 years. In later years they usually adopt other pursuits, such as hunting or the collection from river bush of poles for housebuilding. Girls aged about 7 begin to help their mothers to collect fuel and water from the rivers. At the age of 16 or 17 or earlier they are accepted as brides and from this time onwards regard the collection of fuel and water as a part of their life-long vocation.

Those people who till the soil in or on the fringe of the bush 700 yards from the rivers are in daily contact with the vector practically throughout the year. People living to the west of the Kitare, Sanda and Awach Rivers make frequent visits to the markets of Kodera and Oyugis. The Kodera market is particularly dangerous as it is held at a spot only 50 yards from the infested Awach. During these excursions they cross one or other of the riv   usually delaying at the infested fords to wash, rest and chat.

(b) SURVEY OF BREEDING GROUNDS.

Searches for larvae and pupae were carried out on the tributaries, and on the Sanda and the Awach. Four African assis continuously on this work; their collections were identified, daily. They made collections from rocks in waterfalls, vines, boulders and stones, large and small, in aerated and non-a soon as the vector—*S. neavei*—was discovered, special efforts ing searches in moss, earth and mud of stream banks, to dis places. Despite these efforts the early stages of *S. neavei* we

Species Found.

Pupae of the following species were recorded :—

S. lepidum De Meillon	*S. nigrita*
S. hirsutum Pomeroy	*S. duodeci*
S. alcocki Pomeroy	*S. unicorn*
S. elgonensis Gibbons	*S. damnos*

The indications of these preliminary observations might be summarized as follows :—

1. Adult females of *S. neavei* are active during both hot and cool weather.
2. Dense thicket is not attractive to harbouring or food hunting females.
3. Entirely unshaded spots are avoided by all but a few (probably very hungry) flies.
4. The majority of flies remain within about 300 yards of their probable breeding grounds but a small percentage will travel through light bush as far away as 700 yards.

(vii) *Simulium* in Houses.

Two searchers spent a day searching in huts near the rivers Sanda and Awach. They entered twenty-two huts in all and searched very thoroughly with the aid of hand torches. Not a single *Simulium* adult was found. The local inhabitants, who are aware of the fly, state that it never enters houses.

(viii) Food of *S. neavei*.

Adult females not used for the infectivity examinations were later tested by the precipitin method for the presence of human and bovine blood. No positive reactions were obtained. It is probable that since all captures were of flies that came to the African assistants, all specimens were hungry and not merely curious.

We hope to devise a method of capture for other than hungry flies in the near future.

(ix) *Attempt to Infect Laboratory Animals by the Bite of the Vector.*

Special catches were made along the Kitare River in areas of high infectivity to secure *S. neavei* adults for the purpose of infecting laboratory animals. At first some difficulty was experienced in keeping flies alive during the journey from the river to the camp. But heavy mortality was avoided later by the use of glass cylinders fitted with fine gauze on one end and wet (but not saturated) cotton wool plugs at the other, the cylinder being transported in a lidless wooden box covered with fresh leaves.

One rabbit, one guineapig, two white rats and one monkey were used. All the animals except the monkey were kept in cages measuring 2 feet square. The floor of each cage and one side were of wood, the rest of the cage being of fine muslin. In the wooden side was a door and a circular hole fitted with a cloth sleeve for the purpose of feeding the animals and releasing the adult flies. Owing to its destructive habits the monkey was not kept in a cage; flies were applied to its back for periods of 2 hours in a small glass cylinder with gauze at both ends. In all cases the animals' backs were shaved prior to exposure.

Two hundred and fifty-three female *S. neavei* were released in the cage containing the rabbit and the guineapig and 241 female *S. neavei* were released in the rats' cage. Inspections were made at frequent intervals. The flies were apparently reluctant to feed as they were always seen to be resting on the sides of the cages. The same reluctance was exhibited towards the monkey. Flies were applied to the monkey's back on three successive days but were not observed to bite in one single instance.

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Those people who till the soil in or on the fringe of the bush 700 yards from the rivers are in daily contact with the vector practically throughout the year. People living to the west of the Kitare, Sanda and Awach Rivers make frequent visits to the markets of Kodera and Oyugis. The Kodera market is particularly dangerous as it is held at a spot only 50 yards from the infested Awach. During these excursions they cross one or other of the rivers, usually delaying at the infested fords to wash, rest and chat.

(b) SURVEY OF BREEDING GROUNDS.

Searches for larvae and pupae were carried out on the Kitare River and its tributaries, and on the Sanda and the Awach. Four African assistants were employed continuously on this work; their collections were identified, as far as possible, daily. They made collections from rocks in waterfalls, vines, grasses, tree roots, boulders and stones, large and small, in aerated and non-aerated water. As soon as the vector—*S. neavei*—was discovered, special efforts were made including searches in moss, earth and mud of stream banks, to discover its breeding places. Despite these efforts the early stages of *S. neavei* were not found.

Species Found.

Pupae of the following species were recorded :—

S. lepidum De Meillon	*S. nigritarsus* Coquillet
S. hirsutum Pomeroy	*S. duodecimum* Gibbons
S. alcocki Pomeroy	*S. unicornutum* Pomeroy
S. elgonensis Gibbons	*S. damnosum* Theobald

One new species was discovered which will be described by Dr. B. DE MEILLON at a later date.

S. lepidum was by far the most common species. It was found mostly in turbulent foamy water in the fast flowing portions of the main rivers. A few specimens only were found in the tributaries. *S. hirsutum* was found in slow running water in the main rivers. The other species enumerated above, except the single *S. damnosum* which was found in the Kitare, generally preferred the slow muddy portions of the tributaries.

Breeding Places (see Plate II).

Pupae of *S. lepidum* were present in thousands, attached to rocks and boulders, sticks caught in rapids and rock walls of waterfalls, where water appeared to be well aerated. The presence or absence of shade seemed to be immaterial.

S. hirsutum was quite common but not so common as *S. lepidum*. Most specimens were found attached to vines, grass, leaves of trees, etc., trailing in slow running, non-aerated water at about 1 to 2 inches below the surface and usually though not always in shade. A few specimens were found attached to rocks at about a foot below water surface in turbulent foamy water.

The majority of the pupae of this species appeared to be near the variety *dubium* but Dr. DE MEILLON considers that the adults are typical *hirsutum*.

S. alcocki. Only one specimen was found. It was attached to long grass trailing in densely aerated water cascading over rocks ; in partial shade all day.

S. elgonensis was quite as common as *S. hirsutum* and was found in similar breeding conditions.

S. nigritarsus was found on very few occasions, attached to grass at the sides of streams in turbulent foamy water.

S. duodecimum occurred in small numbers breeding in sluggish water at the side of open sunlit portions of the streams. In every instance it was attached to grass just below the water surface and was buried in the gelatinous muddy substance which enveloped the roots of the grass in these streams.

S. unicornutum. A few specimens were obtained breeding under conditions similar to those of *S. hirsutum*—on grass at the shady sides of streams, just below water level in slow water.

S. damnosum. One pupa only was found together with a number of *S. lepidum* attached to a rock in intensely aerated water at foot of a small waterfall.

The striking points about this survey of breeding places are :—

(*a*) Only one pupa of *S. damnosum* was found.

(*b*) In spite of the large numbers of *S. neavei* and the absence of all other species, except one, in the adult catches, not a single larva or pupa that can be referred to this species was included in collections of several thousands of specimens obtained from a big variety of breeding conditions.

SUMMARY.

1. Onchocerciasis is recorded in an area of South Kavirondo.

2. The skin infection rate in a sample of 609 (men, women and children) was 51 per cent. varying from 98 per cent. to 13 per cent.

3. The vector is *Simulium neavei* which is present in greatest density in areas of highest human infection. The fly infectivity rate for the whole area was 10·1 per cent.

4. Contact between fly and population is made in the vicinity of rivers—in the bush and at fords.

5. *S. neavei* appears to harbour in the lighter bush bordering rivers. It has been found as far as 700 yards from a stream but occurs in greatest numbers within 300 yards.

6. A survey of the rivers and tributaries produced larvae and pupae of *S. lepidum*, *S. hirsutum*, *S. alcocki*, *S. elgonensis*, *S. nigritarsus*, *S. duodecimum*, *S. unicornatum* and one *S. damnosum*, but not that of *S. neavei*.

7. Attempts to infect various animals by the bite of captured adult *S. neavei* appear to be unsuccessful.

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WHAT IS PELLAGRA IN CHILDREN?

BY

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Dr. H. C. TROWELL (1940) has given recently in these TRANSACTIONS an interesting survey of the much discussed condition in children probably caused by malnutrition, accompanied by dermatitis (or should it be " dermatismus " ?) and frequently fatal, which he believes to be " infantile pellagra." It is so rare and refreshing to find a paediatric subject which interests " tropical " physicians, that I venture to draw attention to some other points that are worthy of consideration.

I entirely agree that the cases described by Dr. TROWELL, by PROCTOR, GILLAN and others, including myself, are probably all the same, or are very similar conditions. Unfortunately I have not been able to see all the literature that is quoted.

What's in a name ? It matters very little, as long as we understand each other, and as long as we recognise how much and how little we imply by that name. When it is used to indicate a degree of classification that is unjustified by the present state of our knowledge, then that name is unwise and misleading.

The study of nutrition is still in its crude stages. The extent of its vast complexity is not yet even dimly realized. In some cases of malnutrition we believe we are dealing with one single, or at any rate with one dominant, factor. Efforts at investigation always go to prove that the subject is not as simple as it looked. But in a large proportion of cases the picture is not a clear-cut one representing a single deficiency, it is a complex picture produced by a number of factors acting on that very involved subject, a human organism.

Besides all the unassessed complications of nutritional factors, we also have to deal with the question of assimilation, how it varies in different individuals at different ages, under different conditions, how it is affected by illnesses, by other constituents of the food and by such questions as nervous control. All

these are details which claim our accurate observation, for animal experiment will be of little help.

As medical students we were being constantly warned against a double diagnosis. Now it seems just as necessary to guard against that other snare— the simple explanation.

To regard disease as static and knowledge as final is unwise. There are many conditions that produce skin lesions. Some of these are pellagrous, some more akin to the keratomalacia of a vitamin A defect, and some appear to be non-specific. The same with conditions of the mouth such as glossitis, angular stomatitis, perleche, etc. These are frequently associated with malnutrition. But there are overweight gentlemen who eat, and regretfully assimilate, everything from the caviare to the peaches, who may occasionally be troubled by these symptoms. They may clear up rapidly on A, B_1, B_2, on riboflavin or on nicotinic acid, but very often they clear up for no reason at present ascertainable.

In West Africa sore mouths and glossitis were very common among pregnant women of every class, and whether the main carbohydrate were maize, or roots such as yams and cassava. Palm oil in the diet did not seem to act as a preventive. A balanced diet was always advised, and a mixture containing phosphate and iodide of iron and a nightly dose of calcium lactate seemed to improve the condition. It generally disappeared with the birth of the child. None of these cases developed pellagra or anything resembling it.

The experimental work of TOPPING and FRASER seemed to show clearly that sore mouths would appear in monkeys with any of the known vitamin deficiencies, although the condition was most marked in the absence of the B_2 complex.

Another example. The depigmentation of skin and hair and the straightening of hair that occurs in African children with " infantile pellagra " has been frequently noted. But this is in no way specific. The most marked cases of depigmentation, etc., occur in Africans with the more chronic conditions such as ascariasis, quite apart from any sign of any pellagra whatever. In Singapore we frequently see children with marked oedema of the face and extremities, the hair dry, sparse and staring, but though they may produce large numbers of ascarides (WILLIAMS, 1938) I have only seen one case that developed " infantile pellagra."

The same tendency to classify rather than to observe is typified in many articles and textbooks on nutrition. The constituent percentages of such articles as " human milk " and " rice " are solemnly recorded to two places of decimals. There is no statement of how many readings this is the average, nor of what is the possible range of values. Yet DRUMMOND and his co-workers (1939) found that in twenty-seven samples of human milk the fat percentage varied from 2·4 to 7·1.

It is difficult in the present state of our knowledge even to be sure that " infantile pellagra " is due to a vitamin deficiency. The question of minerals

has not as yet been studied as far as I know. The frequency of the condition along the belt of tropical Africa is suggestive.

The disease is obviously different from classical pellagra. It is almost unknown in infants—cases under one year old are exceedingly uncommon. Three cases were apparently cured with nicotinic acid " in addition to other dietetic measures." Yet Dr. TROWELL boldly opens with " The acute pellagra of malnutrition is only common in, though not restricted to, infants in the tropics."

LANDOR (1939) stated that the cases that he and PALLISTER described in Malaya, and previously classified by some observers as pellagra, did *not* clear up on nicotinic acid.

Dr. D. C. WILSON has kindly permitted me to see some photographs from India. Some of these are typical, classical pellagra. Some I would say, were typical " kwashiorkor " (some looked like a mixture of the two). Dr. WILSON tells me that the condition is of seasonal recurrence. In these cases it was not " acute," it was not infantile, and it did not look like pellagra.

The rash in pellagra is often red and very irritating. In this it is quite unlike that found in " kwashiorkor."

Dr. TROWELL remarks "As there is no unanimity concerning the aetiology and symptoms of the complaint, it has appeared desirable to gather together the different reports which have been issued."

Dr. STANNUS quotes Dr. R. H. TURNER. (1931), " Pellagra was unique in the scarcity of accurate information, etiology uncertain, pathology obscure, diagnosis a matter of opinion, cause of death not understood."

STANNUS (1940) also comments on the diversity of symptoms and aetiology which are described, and the diversity of treatments and the results thereof. His explanation is the ingenious one of different links in the same chain. Rickets ending with a terminal pneumonia are links in a chain of malnutrition, yet one would not call them the same disease nor try to confuse the symtomatology.

LOWE (1931) stated " Possibly some of the confusion and the difference of opinion existing among workers on the subject of pellagra is due to the fact that they are not all talking about the same condition." I submit that he is probably right. He describes forty cases of pellagra on the Deccan. From the description it appears that some of these cases are true pellagra, some may be more akin to " kwashiorkor," and some may be a mixture of the two. His youngest case was 14 years of age.

BLOOM (1928), who is quoted by TROWELL, also remarks that " Infantile pellagra exhibits itself as the most proteiform of all diseases." He describes repeated attacks as the normal event. He regards the facial rash as diagnostic and he does not comment on any very large mortality. He remarks on the frequency with which it is reported in some states, while it is altogether ignored in others.

Is it not likely that these observers are only too correct ? The " lack of unanimity concerning aetiology and symptoms " may possibly be due to the fact that " they are not all talking about the same condition."

Dr. TROWELL has, surprisingly, ignored the work of Dr. R. H. TURNER (1935), another well-known authority in the U.S.A. In his description of pellagra in children in the *Practitioners' Library of Medicine*, Dr. TURNER states that young pellagrins are seldom seen in hospital because the disease in children is such a mild one that it is apt to appear only as a skin condition.

" Probably because children prefer to go out of doors without hats, they are more likely to have skin lesions on the face, especially on the forehead, than are adults. As in the adult, the appearance of pellagra in the child chronically ill of some intestinal disorder which keeps him in bed is likely to be with skin lesions quite insignificant in size and severity. In such patients the skin areas involved are not those commonly sunburned, as is the case for the usual pellagrin who is out of doors during the days of onset, but in such areas as between the folds of the buttocks, over the pubis and the points of the elbows. . . . Ulceration may occur. In any child who shows diarrhoea and sore mouth, the skin should be searched for dermatitis. . . . Vaginitis does not appear to be of so much importance in children. . . . The child who has had repeated attacks of pellagra is likely to show arrest of mental development. . . . In children the prognosis of pellagra is vastly better than in adults." Possibly in this account also, two conditions are being described.

In 1936 I had the pleasure of visiting New Orleans, and the privilege of discussing the subject with authorities such as Dr. ROBERT STRONG, Dr. C. C. BASS and Dr. R. H. TURNER. It is tentatively and prepared for correction that I refer to these discussions, but the impression I received was :—

(1) The syndrome we see in Africa is rare in its acute form in the Southern States.

(2) Classical pellagra is seen in adults. It is comparatively rare, but by no means unknown, in infants and children.

(3) It is almost invariably accompanied by a rash on the face.

(4) It is a comparatively mild condition in children, and responds to treatment more readily than in adults, although relapses are known to occur.

Permission was given for me to examine the records in the Charity Hospital, New Orleans. It appears that, among adults (patients over 12 years of age), pellagra was diagnosed in eighteen cases during the 9 months, January to September, 1936, while in the 5 years, 1931-36, there were nine cases of pellagra in children. The youngest of these, and the only one that died, was 20 months of age. The others were 6, 11, 11, 7, 10, 6 and 7.

Dr. ROBERT STRONG has very kindly given permission to reproduce some of the photographs of pellagra in children from the museum of Tulane University (Figs. 1, 2 and 3) as well as the photograph of severe pellagra in a negro child

FIG. 1.—Pellagra in a child.
(Museum of Tulane University.)

FIG. 2.—Pellagra in a child.
(Museum of Tulane University.)

Fig. 3.—Pellagra in an Egyptian child. (Museum of Tulane University.)

Fig. 4.—Pellagra in a Negro child.
(Dr. R. A. Strong's case.)

FIG. 5.—" Kwashiorkor " in a Chinese child. Child died of tuberculosis.
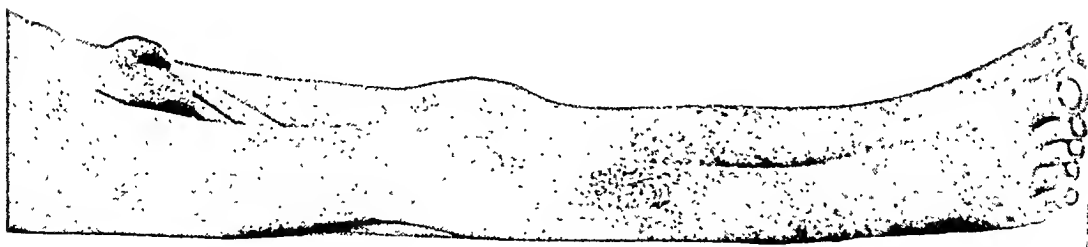
FIG. 6.—Nutritional rash in a Chinese child. Some oedema of feet. Child dying of tuberculosis.

from a case of his own (Fig. 4). This child had desquamation of the buttocks and bedsores on the sacrum and ultimately died.

Another point that is worthy of note is the rarity of classical pellagra in Malaya. It is probable that B_1 and B_2 occur together in many foodstuffs, yet, in Singapore where B_1 deficiency is only too common, where beriberi is one of the most serious causes of mortality and morbidity among every age of the population, yet pellagra is rare in adults and unknown in children. Nor have I seen " kwashiorkor " except in one case. A child of 4 was admitted in a typhoid-like state. His father kept a shop, and the diet he described was a good mixed one. The child developed the rash of " kwashiorkor " in its most blatant form (Fig. 5), and it cleared up with A, B_1, nicotinic acid and all the other nutritional adjuvants we could remember. The child had had ascariasis. In spite of the improvement in the skin the child died. Postmortem the liver appeared normal but there was massive pulmonary tuberculosis.

A rash on the extensor surface of the tibia is commonly found in a large proportion of cases of malnutrition. A well marked case is shown (Fig. 6). This child of 4 years old had been sick for one year, and for the last 5 months he had been exclusively fed on sweetened condensed milk. He died a few hours after admission. Postmortem in this case also, there was found extensive pulmonary tuberculosis.

On one occasion I went over 160 beds at the Singapore General Hospital, for women and children, mostly of the coolie class. On forty of these cases one was able to demonstrate some pigmentation, dryness and or desquamation on the anterior aspect of the tibia. The ages varied from a few months to 60 years. The diseases for which they were admitted were malnutrition, beriberi, typhoid, tuberculosis, enteritis, thyrotoxicosis, senility, peritonitis, pneumonia, etc., etc. In some cases there were patches of associated phrynodermia. in some there was evidence of glossitis and or perleche, but not one of them could have been called pellagra by classical standards, and none of them, or of their kind, ever developed pellagra. Nor, although they started in the same way, did they ever develop a full-blown " kwashiorkor."

This rash on the anterior aspect of the tibia is probably another non-specific manifestation of malnutrition. or perhaps it is what has been called keratomalacia (whatever that may be).

In conclusion I venture to suggest that many and various are the forms of malnutrition, and many and various are the skin conditions arising therefrom. There is nothing to prevent a mixture of nutritional diseases in the same individual. hence the appearance of many puzzling and indeterminate conditions. Until the biochemistry of health and of disease has been studied with far more detail and knowledge than we have at present, it is unwise and confusing to the issue to label a variety of symptoms with a name that implies an as yet unproved identity.

If the photographs are considered in conjunction with those previously

published, I think it should be conceded that, in its well developed form, pellagra in children has a different appearance from that of "infantile pellagra."

Until the subject has been further elucidated, the name pellagra had better be reserved for the classical condition, while any other dermatitis which is suspected of a nutritional antecedent we might, as a compromise, refer to as the Pellagroid of Procrustes.

I am very much indebted to the various authorities at the Charity Hospital and at Tulane University for their courtesy and for facilities to make investigations, particularly to Dr. ROBERT A. STRONG for permission to use the photographs.

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YELLOW FEVER IN BRITISH GUIANA.

FURTHER OBSERVATIONS.

BY

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In a previous communication (SNEATH, 1939) the distribution of immunity to yellow fever was reported from random sampling of residents in the hinterland of the Colony. Representation of the younger age groups was notably deficient and the fewness of blood samples from certain areas was such as to warrant an extension of the collection. Although these deficiencies are not entirely rectified it has been possible to extend the observations by 217 specimens obtained from nine previously designated areas. Mouse protection tests

were undertaken in Rio de Janeiro through the continued courtesy of the International Health Division of the Rockefeller Foundation, and 59 sera (27 per cent.) were found to be immune. Approximately 10 per cent. of the total sera that have been tested were found to show *inconclusive* or *unsatisfactory* results. These have been considered as *negative* in compiling the data in this as well as the previous report. Since it is possible that a closer analysis of the *inconclusive* tests might indicate a greater proportion of naturally immunised persons, it is submitted that these findings may be considered as conservative.

AREAS.	Area 1. N.W. Kanaku Foothills. T.	I. No.	Per cent.	Area 2. S. Kanaku Foothills. T.	I. No.	Per cent.	Area 3. Northern Rupununi. T.	I. No.	Per cent.	Area 4. Southern Rupununi. T.	I. No.	Per cent.	Area 5. Hinterland. Essequebo Coast. T.	I. No.	Per cent.
1–4	—	—		11	1		2	1		—	—		—	—	
5–9	—	—		8	2		2	2		—	—		3	—	
10–14	1	—		3	2		4	1		7	5		2	—	
15–19	3	—		6	2		4	2		7	3		4	—	
20–29	—	—		1	—		10	6		17	4		10	1	
30–39	—	—		3	—		2	1		3	1		1	—	
40+	1	—		1	—		1	1		8	6		1	1	
Totals	5	—	—	33	7	21	25	14	56	42	19	45	21	2	9
Previous Report Totals	61	13	21	34	19	56	91	60	66	6	2	33	41	11	27
Summary	66	13	20	67	26	37	116	74	64	48	21	44	62	13	21

T = Total blood specimens;   I = Immune sera; . *= Obtained from

In view of the observation that natural infection in Brazil appears to induce a clear-cut distinction between immune and non-immune sera in contrast to the greater indefiniteness to be found in persons who have been actively immunised with the attenuated virus 17D (SOPER, 1938) one cannot fail to wonder whether it is possible that the natural virus responsible for the immunity in this Colony may have inferior antigenic properties to that occurring in Brazil, thus resembling the attenuated virus in this respect. The question of whether the virulence of the local virus by analogy may be considered as being less than

that within the Brazilian experience, is something upon which we have no evidence.

The Table has been compiled to show the age-group distribution of the additional sampling in the same areas previously reported upon, in order that comparison may be possible.

Comment on Table.

(1) The few additional sera included in Area 1 do not materially alter the

Area 6.		Area 7.		Area 8.		Area 9.		Summary.		Grand Total.	
North-West District.		Mazaruni Head Waters.		Potaro, Mazaruni and Cuyuni Valleys.		Orealla Indian Reserve.					
I.		T.	I.	T.	I.	T.	I.	T.	I.	T.	I.
No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
—		1	—	—	—	—	—	14	2	14	14	2	14·3
—		1	—	—	—	2	—	16	4	25	16	4	25·0
—		2	1	17*	—	18	—	54	9	17	62	11	17·7
—		2	1	—	—	—	—	29	7	24	64	17	26·6
3		11	2	3	1	—	—	61	17	28	161	55	34·2
2		2	2	2	1	—	—	20	7	35	89	36	40·5
2		1	1	4	2	—	—	23	13	56	100	60	60·0
7	28	20	7	35	26	4	15	20	—	—	217	59	27
1	25	11	7	61	6	3	50	19	5	26	289	126	44
	25	53	14	42	32	7	22	39	5	13	506	185	37

* ... of Bartica at confluence of the above rivers with Essequebo River.

significance of the previous findings. The importance of this area in the general array appears to be secondary.

(2) Although the proportion of immune persons in Area 2 is considerably lower than in the previous report, this one includes a reasonably representative sampling of the younger age groups and as such gives clear evidence that the area is an active focus of infection. It is also evident that the proportion of immune persons increases progressively with age from early childhood. Proportionately this area is of primary epidemiological importance.

(3) The proportion of immunes found in Area 3 is the highest in the whole array, but this is probably explained by the disproportionately large numbers of sera obtained from the age groups from 20 years on. The situation indicated by the immune children was noted in a postscript to the previous report and requires no further comment.

In a pamphlet issued by the SURGEON-GENERAL in 1925 on *Scurvy in British Guiana* it was stated: "During 1924 a severe epidemic of scurvy was reported in the Rupununi and Berbice balata districts ... and no less than sixty-five deaths are reported to have occurred. The disease also occurred in the diamond and gold mining fields." Since it seems probable that this diagnosis may have been made as a result of hearsay evidence, as it might be today in the absence of clinical observation by a medical officer, in consequence of the contemporary evidence that jungle fever is prevalent in this area, it is possible that the haemorrhagic signs of disease associated with the deaths considered as scurvy might have been a clinical indication of the jungle form of yellow fever. Because such a high proportion of the surviving balata-bleeders of that time who are still engaged in this occupation in Area 3 are immune to yellow fever, this hypothesis may bear consideration as an indication of the appearance of jungle fever in the Colony. Should this be tenable it would merely confirm the presumption that this area has been of primary importance for a great number of years and that balata-bleeders as a class are particularly exposed to jungle fever. Miners ("pork-knockers") as a class have not been surveyed in sufficiently significant numbers to indicate the probable extent of their exposure.

(4) For the reason that the collector was a stranger to the parents of children in Area 4, it was not possible to obtain blood from the youngest age groups. However, from the augmented sampling it would appear that epidemiologically this area does not differ from Areas 2 and 3 in importance.

(5) From the limited representation of the younger age groups in Area 5 it may be justifiable to suspend judgment as to the contemporary focal import of this area. Taken in conjunction with the previous records it is probable that this may be of secondary importance as a source of infection, but from the proportional distribution of immunes by age groups, it cannot be doubted that the disease occurs here.

(6) It has not yet been possible to obtain sampling of the younger age groups in Area 6, but from the limited evidence submitted it appears probable that this area may be of secondary importance.

(7) By fortuitous circumstances it was possible to extend the blood sampling from inhabitants of Area 7. This area because of its remoteness is probably of least significance as a source of infection to the coastlands, but in view of the age distribution of immune persons is doubtless of equal importance as a focus of infection to Areas 2, 3 and 4.

(8) The reports from Area 8 are overweighted by a group of negative sera obtained from children resident in a town (Bartica) at the confluence of the river valleys concerned. The sera from adults allocated to this area have been obtained from persons engaged in the mining and timber industries more or less remote from this town. So far it has not been possible to obtain a satisfactory sampling of residents in these valleys. Because of the distribution of immunes in Areas 3, 5 and 7 between which this area lies, it is probable that the incidence of the disease here is very similar. Since the town noted is so situated as to be exposed to the introduction of infection from Area 3, as well as from the remainder of Area 8, the non-immune status of resident children of the age group 10 to 14 years indicates their potential vulnerability and that of the other residents in the event of the disease being introduced to that town from these hinterland foci.

(9) Finally, in the previous report it appeared that the Indian Reserve, Area 9, had experienced yellow fever within the previous 15 years. A further sampling was undertaken of the population in the age group 5 to 14 years. From the non-immune status of these it is evident that exposure to yellow fever has not occurred during the lifetime of this group of children. The location of this Reserve outside the forested belt of the Colony suggested an anomaly for which no explanation was offered in the first report. From the information now available it appears probable that as in the Rupununi savannahs such immunity as may be acquired in this place must be a consequence of exposure within the forested area to which the younger age groups are not liable because of the distances involved. There is no valid reason to consider this area as a focus of infection at the present time.

SUMMARY.

With an additional blood sampling from the hinterland of British Guiana it is considered justifiable to conclude that certain areas of the Colony have greater importance as foci of the jungle form of yellow fever than others. The inclusion of representatives of the younger age groups in this sampling makes it clear that this disease is endemic in the hinterland of the Colony.

Inspector SINGH of the Health Branch of the Department. It must be noted that without the facilities for mouse-protection tests generously undertaken by the Rio Laboratories of the Rockefeller Foundation, these reports would have no significance. Further, I have to thank the Director of Medical Services, British Guiana, for permission to publish this report.

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YELLOW FEVER IMMUNE BODIES IN SHEEP SERA.

BY

E. C. SMITH, M.D., M.R.C.P.*

Medical Research Institute, Lagos, Nigeria.

The presence of yellow fever immune bodies in the sera of mammals from Africa and elsewhere has been established and the possibility that in addition to the primates, other animals, both wild and domestic, may serve as a reservoir for the virus of this disease has been suggested.

In this respect considerable attention has been paid to cattle and sheep; and MacCallum and Findlay (1937), have recorded their findings with sera obtained from these animals in various parts of Africa.

Of 135 sera, collected from the Anglo-Egyptian Sudan, Sierra Leone, Uganda and Kenya, 19 per cent. gave positive results with the mouse protection test.

Of eighty-two cow sera from Kenya, eleven were positive. So far as it is known, no human cases of yellow fever have been recorded nor have positive results been obtained with protection tests on human sera from this country.

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Of eighty-two cow sera from Kenya, eleven were positive. So far as it is known, no human cases of yellow fever have been recorded nor have positive results been obtained with protection tests on human sera from this country.

The authors, however, point out that the possibility of these animals being bitten by mosquitoes or other arthropods infected with the virus of yellow fever cannot be excluded. The same authors report three positive results in seventy sheep sera from Kenya. As a control they examined 119 sera obtained from cows in England, one animal gave a positive result but when examined again 5 months later the serum was negative.

An attempt to infect a calf with a viscerotropic strain of yellow fever virus did not give rise to any symptoms but when the serum from this animal was tested for immune bodies after an interval of 21 days a positive result was obtained.

SALEUN (1938) who confirmed these observations found that of fifteen cows from the region of Lake Tchad ten gave a negative sero-protection test for immune bodies to yellow fever while five gave a positive reaction, one in high dilution.

The present paper records the findings in connection with sera obtained from local sheep and includes the results of certain inoculation experiments, using the neurotropic strain of the virus.

Sheep are used at the Medical Research Institute, Lagos, for the production of anti-variola and anti-rabies vaccines. The animals are obtained from Kano (Northern Nigeria) and during 1939 one hundred sera were submitted to the mouse protection test. The technique employed is the same as that described by SAWYER and LLOYD (1931) and the results are presented in tabular form.

TABLE I.

No. of sheep sera.	Negative. 0-1 mouse surviving at 10 days.	Doubtful. 2/6 mice surviving at 10 days.	Partial protection. 3-4 mice surviving at 10 days.	Full protection. 5-6 mice surviving at 10 days.
100	53	15	16	16

INOCULATION EXPERIMENTS.

In order to discover whether the local sheep were susceptible or not to the virus of yellow fever the following experiments were carried out.

Since MACCALLUM and FINDLAY (1937) were able to demonstrate the presence of virus in the peripheral blood of the cow inoculated by them strict precautions were taken to prevent mosquito transmission.

A. SERIAL INTRA-CEREBRAL INOCULATIONS.

Two sheep (Table II, Sheep 1 and 2) the sera of which were previously tested for immune bodies to yellow fever and found to be negative, were inoculated intracerebrally with 0·5 c.c. of a 12 per cent. suspension of a neurotropic yellow fever virus, lethal for mice in 3 days. On the fourth day Sheep 1 was killed by severing the carotid artery. A portion of the cerebrum

was removed with aseptic precautions, suspended in saline (approximate dilution 1 in 5) and 0·5 c.c. was inoculated intracerebrally into two non-immune sheep (Table II, Sheep 3 and 4). The same suspension, diluted 1 in 10 was inoculated also intracerebrally into six white mice. Sheep 3 developed diarrhoea and had to be destroyed on the 3rd day after inoculation.

Sheep 4 was killed on the 6th day.

A portion of the cerebrum was removed, suspended in saline (approximate dilution 1 in 5) and 0·5 c.c. was inoculated into two non-immune sheep (Table II, Sheep 5 and 6). Six white mice were inoculated as before using a 1 in 10 dilution of the suspension.

Five days after the inoculation, Sheep 6 was killed and a portion of the cerebrum was used for passage into two more sheep (Table II, Sheep 7 and 8) and into six white mice.

Sheep 7 developed pneumonia and died on the 20th day.

None of the sheep so far inoculated exhibited symptoms suggestive of a cerebral infection with the exception of Sheep 5. On the 12th day after inoculation this animal developed symptoms which were very similar to those observed in sheep inoculated intracerebrally with rabies virus and consisted of loss of appetite, weakness and some spasticity of the posterior extremities, generalized tremors and increasing inability to maintain the head in an upright position. There was no rise in temperature. Later the paralysis became generalized, the animal was unable to stand and it was killed in the usual manner. A portion of the cerebrum was removed and 0·5 c.c. of a 1 in 5 suspension in saline was inoculated into Sheep 9 (Table II). Six white mice were inoculated also using a 1 in 10 suspension.

Sheep 9 developed symptoms on the 10th day and a further passage was made (Table II, Sheep 10). The latter developed symptoms on the 13th day. No suitable sheep were available for transfer but six mice were inoculated with a 1 in 10 suspension of brain in saline.

The results of the inoculations in mice are shown in Table II.

B. NON-SERIAL INTRACEREBRAL INOCULATIONS.

Six sheep, the sera of which gave negative results with the mouse protection test were inoculated intracerebrally with 0·5 c.c. of neurotropic (mouse) virus.

Two of these sheep did not develop any symptoms, one was found dead on the fourth day and as postmortem changes had set in no examination was carried out. One animal died on the 22nd day after inoculation and subsequent examination revealed the presence of an advanced bilharzial infection of the liver. Of the two remaining animals, one developed paralytic symptoms on the 9th day and the other on the 13th day. The condition of both became rapidly worse and the animals were killed. A passage was made into mice in each

TABLE II.

SERIAL INTRACEREBRAL INOCULATIONS.

Neurotropic Virus

S.1   S.2—No symptoms
                                          F.P. at 30 days.

Passaged at
4th day

S.3   S.4   M¹

Discarded 3rd day   Sick at 5th day

                                                  M²

                    Passaged at   Sick at 3rd day
                    6th day

S.5   S.6   M¹

                                              Sick at 5th day

                    Passaged at   M²
                    5th day

            S.7   S.8   M¹—— Sick at 5th day.   Sick at 3rd day

                          No symptoms
                          N.P. at 60 days

        Pneumonia
        20th day

Symptoms
12th day

S.9   M¹

                    Sick at 5th day

Symptoms   M²
10th day

                    Sick at 3rd day

S.10   M³

                    Sick at 3rd day

Symptoms   M⁴
13th day

M¹   Sick at 3rd day

                    M⁵

                    Sick at 3rd day

Sick at 5th day   M⁶

                    Sick at 3rd day

M²   P.T.

Sick at Third day

M   = Mouse brain passage.
S.   = Sheep brain passage.
N.P. = Negative protection test.
F.P. = Full protection.
P.T. = Protection test (to confirm
          presence of yellow fever
          virus).

case and portion of the brains and livers were preserved for histological examination.

<div align="center">C. SUBCUTANEOUS INOCULATIONS.</div>

Two non-immune sheep were inoculated subcutaneously with 5 c.c. of a 12 per cent. emulsion of neurotropic mouse virus. No symptoms developed over a period of 2 months.

Samples of blood were obtained from two sheep in the first series of intracerebral inoculations (Table II, Sheep 2 and 8) ; from the two in the second series which remained normal and from the two which were inoculated subcutaneously. The time interval varied from 15 days to 2 months from the date of inoculation.

All the sera with the exception of Sheep 8 gave full protection with yellow fever virus in mice. Sheep 8 was negative and it is to be presumed that this animal was non-susceptible.

Throughout the experiments the usual control cultures in broth, with subcultures into nutrient and blood agar were made and were negative.

A general postmortem examination was made on all the sheep which were killed or which died (with the one exception stated) in order to exclude other infections.

<div align="center">MORBID HISTOLOGY.</div>

Paraffin sections of the brain and liver of Sheep 5, 9 and 10 of the serial group and of the two animals of the non-serial group which developed symptoms were prepared and stained by haematoxylin and eosin. In addition, frozen sections were prepared from the liver of each and stained for fat (Sharlach R).

<div align="center">*Brain.*</div>

Sections from Sheep 9 and 10 showed marked congestion of the vessels with well defined perivascular cuffing.

Diffuse infiltration of the tissue with round cells was also noted. Sections of the brains of the remaining animals showed little beyond vascular congestion and occasional foci of scanty round cell infiltration.

<div align="center">*Liver.*</div>

Fat stain.—Well defined scattered areas of fatty changes were seen in the preparations from the first series of sheep (Sheep 5, 9 and 10). The sections from the livers of the remaining two animals showed only isolated fat globules such as might be found in a normal liver.

Haematoxylin eosin stain.—Areas of necrotic hepatic cells with haemorrhages were noted in the sections from Sheep 9 and 10 and to a less extent in Sheep 5.

The liver sections from the non-serial group showed nothing abnormal.

As a further control to the findings observed in the livers from the first group (Sheep 9 and 10) sections were prepared from three apparently normal sheep and from two which had been used for anti-rabies vaccine production. Mild fat changes (scattered isolated globules) were present in all, but no necrotic areas or haemorrhages were seen.

The experiments described show that neurotropic yellow fever virus is able to survive for many days in sheep brain tissue and that it is capable of causing death after serial passage.

That the neurotropic virus is capable of causing a fatal infection by direct inoculation is not so certain.   The fact that two animals succumbed to this method of inoculation cannot be taken as conclusive proof in the absence of definite histological findings. It is possible that they may have died from some unrecognized intercurrent condition. The recovery of the virus from the brain by mouse passage is not in itself conclusive but merely serves to confirm the findings recorded in the first series of experiments with regard to its longevity in sheep brain tissue.

The neurotropic virus, though capable of producing immunity when inoculated subcutaneously in two sheep did not give rise to an infection.   In this respect it is of interest to note that SMITH (1936) showed that the Nigerian hedgehog was susceptible to the neurotropic strain of yellow fever virus when inoculated intracerebrally but that this animal did not react to subcutaneous inoculations.

FINDLAY and CLARK (1934), however, using neurotropic virus were able to infect the European hedgehog by both routes.

DISCUSSION.

The percentage of Nigerian sheep, which have been shown to contain yellow fever immune bodies in their blood is considerably higher than that recorded by MACCALLUM and FINDLAY (1937) in connection with sheep from Kenya and it seems reasonable to assume that the immunity is an acquired and not a natural one.

It would be necessary, however, to carry out examinations on much larger numbers of sheep (also cattle) both from areas where yellow fever is unknown and where it is endemic.   Valuable information might be obtained, too, from an examination of the sera of sheep and cattle from various parts of Nigeria and from comparison of the findings with the results of surveys of human sera such as those which have been recorded by BEEUWKES and MAHAFFY (1934).

If the presence of immune bodies in the sheep sera is admitted to be due to an acquired infection one has to consider the possibility of diseases other than yellow fever giving rise to the immunity. Sufficient work has not yet been done

to definitely exclude the production of non-specific immune bodies by diseases of sheep and cattle, and close co-operation with the Veterinary Department is here indicated.

Rinderpest, common in Nigeria, does not seem to require consideration in this respect in view of the statement by MacCallum and Findlay (1937) to the effect that many of the African cow sera examined by them had been previously immunized against this disease.

Sheep are used at the Institute for the production of vaccine for two virus diseases, i.e., smallpox and rabies. Two of the sheep whose sera had given a negative protection test were inoculated cutaneously and intra-cerebrally with vaccinia and rabies virus respectively.

The sera were re-examined after an interval of 7 days in the case of the rabies inoculation (animal moribund) and after 30 days from the appearance of the vesicular eruption in the case of the sheep inoculated with vaccinia virus. Both sera gave negative results.

The fact that neurotropic yellow fever virus can survive for long periods in sheep brain tissue, and that in serial passage it is capable of producing an infection supports the suggestion that animals other than the primates, may constitute a hitherto unrecognized reservoir of the virus.

In view of the strict vegetarian diet of sheep, infection by the alimentary tract by non-biting arthropods can be excluded and the mode of transmission is limited to the mosquito or some other biting arthropod.

It must be noted, however, that spontaneous yellow fever in monkeys has been recorded by Findlay and MacCallum (1939).

It will be seen from Table II that the virus, after passage in sheep, is attenuated to some extent for mice since a longer interval elapses before death occurs in the latter.

The attenuation would probably increase after prolonged serial passage in sheep and with the stabilization of the virus in these animals they might conceivably be used for large scale vaccine production.

The inoculation of sheep with viscerotropic yellow fever virus was not attempted as it was considered inadvisable to maintain a strain at the Institute owing to the danger of mosquito transmission.

CONCLUSIONS.

1. Of 100 sheep sera from Northern Nigeria, thirty-two gave positive results with the mouse protection test.

2. Neurotropic yellow fever virus can survive for at least 13 days in the brain tissue of sheep.

3. By serial intra-cerebral inoculations with neurotropic virus it is possible to produce a fatal infection in sheep.

4. Intracerebral and subcutaneous inoculations bring about an immunity to yellow fever as shown by the mouse protection test.

5. Following the passage of the neurotropic virus in sheep brain tissue its action in mice is retarded for one or more transfers.

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SULPHANILAMIDE IN THE THERAPY OF TROPICAL ULCER.

BY

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The prolonged disability produced by tropical ulcer (*i.e.*, the ulcer in which *Treponema vincenti* and *Bacillus hastilis* are found in association) and the poor response of this condition to out-patient medical treatment led me to explore the possibilities of sulphanilamide in this disease.

As has been shown by CORKILL (1939) the therapeutic response shown by tropical ulcer depends on its age ; and in order to compare results I have divided my cases into three groups—long standing, recent, and pre-ulcerative.

The administration of sulphanilamide by mouth did not, of course, obviate the use of appropriate local applications.   These applications were of two types : those designed to clean dirty ulcers, including magnesium sulphate and glycerine, copper sulphate solution (GUNTER, 1938) and those which promote epithelialization—cod liver oil, whale oil and scarlet red sulphonate.

Long-standing Ulcers.

In this type of ulcer the margin is thickened, heaped-up and fibrous whilst the base is often comparatively clean.   As shown below, the results of treatment of ulcers in this stage with sulphanilamide compounds are uncertain and unsatisfactory.   This may be due to the fact that the organisms keeping the ulcer open are largely saprophytic and therefore uninfluenced by this drug, which, according to McINTOSH and WHITBY (1939), are most active on highly virulent organisms.

Eight cases of this type were treated.   In six cases, M. & B. 693 (2-sulphanilylamino-pyridine) was used, the daily dosage being 3·0 gramme, except in the case of a child which received 1·5 gramme daily.   Prontylin (p-aminobenzene-sulphonamide) 1·8 gramme daily was given to the remaining two.   Of the cases treated with M. & B. 693, only two showed a satisfactory response (i.e., were healed within 3 weeks), these included a child receiving half the normal dosage.

Recent Ulcers.

Under this heading are included ulcers of not more than 1 month's standing.   Clinically they tend to have less heaped-up margins, the base is ragged and exuding a purulent exudate and they are more painful than the chronic type.

Of ulcers of this type, thirty-eight cases were treated (thirty-five cases received M. & B. 693 and three received prontylin), and of these fourteen cases were favourably influenced : twelve of these had been given M. & B. 693 and two had received prontylin. Of the cases favourably influenced, local applications had been made as follows : Copper sulphate solution (1 in 150), four cases ; magnesium sulphate and glycerine, three cases ; cod liver oil, two cases ; scarlet red sulphonate, one case.

All these cases healed within 2 weeks.

Pre-ulcerative Condition.

This is a minute vesicle and often appears to arise spontaneously (LOEWENTHAL, 1932 ; CONNELL and BUCHANAN, 1933). However, in many of my cases I was unable to exclude the possibility of biting insects, the commonest being *Culicoides amazonius*, *C. furens*, *C. stellifer* and *C. diabolicus* (MYERS, 1935 ; ADAMSON, 1939), or of *bête rouge* (*Leptus batatas*) which, as shown by BRUMPT (1922), is capable of producing severe ulceration of the legs. In these types of ulceration which follow bites, there may be an initial vesicle which closely resembles that of the idiopathic type.

The pre-ulcerative stage was only seen in European women and children who had received adequate, vitamin-rich diets. That it was not seen in negroes or East Indians was probably due to the fact that these patients do not bother to seek medical advice until ulceration is well advanced.

In all the pre-ulcerative cases (twelve in number) the disease was arrested and actual ulceration never occurred, but it must here be remarked that ulceration might not have occurred had sulphanilamide been omitted.

PROPHYLACTIC USE.

Limited supplies of the drug prevented my testing its possibilities as a prophylactic. In oilfield practice heavy blows to the lower extremities producing extensive bruising or tearing of tissues often give rise to slow-healing ulcerations and it would be of interest to note if any reduction in this type of complication would be produced by routine administration of sulphanilamide compounds to all recent cases of leg-trauma.

DISCUSSION.

Good effects on tropical ulcer, following sulphanilamide therapy, have been reported by BAYLEY (1939) but MANSON-BAHR (1939) describes two cases in which it had no effect. A case of " gangrenous erysipelas " (érysipèle disséquant), complicating kala-azar, in which a favourable influence was exercised by rubiazal (carboxysulphamido-chrysoidine) is described by BENHAMOU (1937) but the causative organism in this case appears to be *Bacillus terebrans*.

In my series of cases, the action of sulphanilamide compounds would appear to be doubtful in the case of long standing ulcers, favourable in the case of recent ulcers and good in the pre-ulcerative or vesicular state. The value of the drug as a prophylactic in this condition remains to be proved.

SUMMARY.

1. A series of cases of tropical ulcer treated with sulphanilamide derivatives, is described.

2. The difference in action of the drug depending on the state of development of the ulcer, is indicated.

3. The possibilities of prophylaxis with this compound is pointed out.

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NOTES ON THE PREPARATION OF CERTAIN MEDICINAL SOLUTIONS FOR INTRAVENOUS INJECTIONS AND ENEMATA USED IN · THE TROPICS.

BY

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In tropical practice and in the routine treatment of patients, such as those admitted to the Hospital for Tropical Diseases, London, medicinal enemata are commonly used, specific drugs have frequently to be injected intravenously, and physiological or hypertonic saline, isotonic glucose solution or alkaline fluids introduced *via* the veins. The following notes were compiled in the first instance after the writer was asked for information on the dispensing of such preparations and it was subsequently thought that details of the methods used might also be of interest to tropical practitioners.

Many of the more recently synthesized specific drugs, such as stibophen (fouadin) are put on the market in sterile solutions suitable for immediate injection. Others, such as neostibosan, neostam (the nitrogen glucoside of aminophenylamino stibonic acid) ; suramin (Bayer 205, antrypol) ; and tryparsone (tryparsamide) have merely to be dissolved in a stated volume of sterile distilled water before injection. Even where such solutions are stable it is preferable that the drug in solution should be dispensed on the day on which

the injection is to be given. At the above hospital they were injected within an hour or two of their being dispensed and were never allowed to stand overnight. Antimony sodium tartrate, which is so extensively used in the treatment of schistosomiasis and other tropical diseases, may be cited as an example of such a drug.

I.—INTRAVENOUS INJECTIONS.

Distilled water forms the solvent used in all intravenous therapy, and if pyrexial reactions are to be avoided when the more bulky injections are employed, it is essential to have available pyrogen-free distilled water for intravenous use.

The Preparation of Pyrogen-Free Distilled Water.

Freshly-prepared double-distilled water must be used, prepared the same day for all injections exceeding 10 c.c. in volume, and in any case not more than 24 hours after distillation. The type of still should be a pyrex all-glass apparatus consisting of a distilling flask with a long neck (to prevent the carrying over of droplets into the distillate) and a side-arm which fits into the ground-glass joint of a Liebig condenser. The water should be led from the condenser to the receiving flask along a glass tube fitted at the delivery end with a glass hood fused to the tube ; thus the water is not exposed to the atmosphere. Should such a fitting not be obtainable, corks made of a special sulphur-free rubber, and bored to the diameter of the delivery tube, have proved quite satisfactory at the above hospital. This apparatus is necessarily smaller than the usual still, but a 3-litre distilling flask, with either a single-surface condenser (length of outer jacket 25 inches), or, for compactness and the saving of space, a double surface condenser, will deliver about 1 pint every hour on reaching boiling point.*

Glass beads or rods of capillary tubing with open ends should be kept in the distilling flask to avoid " bumping ". The whole apparatus should be cleaned at least weekly, first with alkali and then with acid, and rinsed very thoroughly with successive amounts of water, and finally with distilled water. Before use after cleaning, the distilling flask should be rinsed with pyrogen-free double-distilled water, and filled not more than three-quarters full with single-distilled water prepared in the usual type of still. The first 200 c.c. of the distillate should be rejected.

These precautions can be taken even in a small dispensary to avoid the possibility of rigor after injection in patients, who, having lived some time in the tropics, often present difficulties not met with in those who live in more temperate climates.

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*An emergency method has been described by CLARK et al. (1940), in the British Medical Journal, March 16, p. 430.

1. ANTIMONY SODIUM TARTRATE.

This was found to be less toxic and irritant than the potassium compound (tartar emetic). A number of fatal cases have been reported following the use of old preparations put up in rubber-capped bottles. As this compound is one of the few which can be sterilized in solution by boiling, it affords a cheap as well as an effective treatment. It can be obtained in crystalline form. The solutions for injection should be made individually, a convenient and cheap flask being the Erlenmeyer (30 c.c. with a wide neck). After weighing out the drug, dissolving it in more than the required volume (usually 10 c.c.), and filtering it into the flask, the solution is sterilized by gently boiling down to the 10 c.c. mark on the graduated flask. Sterile ampoules are on the market, but, where economy is an important factor, this is one of the relatively few opportunities of exercising it, without detriment to the preparation.

It might be said in passing that the plugs used for closing the flasks were sterilized before use and were made of non-absorbent cotton wool enclosed in ribbon gauze.

2. QUININE DIHYDROCHLORIDE.

Ampoules of varying strengths are obtainable for intramuscular and intravenous injection, but it has been found better never to administer this drug in a concentration exceeding 1 grain in 1 c.c.

When it is necessary to prepare sterile solutions in the dispensary the best colourless quinine acid hydrochloride should be dissolved in freshly prepared double-distilled water. The solution should then be filtered through a Berkefeld filter, filled into ampoules and sterilized by boiling once in a water bath for half-an-hour. A concentrated solution may be prepared merely for convenience. It has no advantage over the weaker solution as to keeping properties. Such a solution should keep for a considerable time as it is not easily contaminated because of the nature of the drug, which tends to be self-sterilizing.

Should a number of injections be required on any one day, and should ampoules not be obtainable, the solution can be sterilized on a water bath in an ordinary sterile boiling flask having a sterile plug. A suitable size might be a flask with a boiling capacity of 100 c.c. If only a part of the solution is removed from the flask for injection, it would not appear to be necessary to re-sterilize the remainder, as the solution darkens in colour on successive boilings, and in any case tends to remain sterile. Should the physician order a second boiling there would, however, be no objection to this as the quinine salt is stable.

An alternative method described by HOWARD and CHICK* consists in sterilizing the quinine dihydrochloride by steam at 10 lb. pressure (*i.e.*, 24 lb. in all) for 30 minutes ; this does not harm the quinine salt.

Pharm. J. (1917). 2, 143.

3. Sodium bicarbonate solution.

(150 grains to the pint.)

This solution is sometimes required for intravenous injection in relatively large volume. It cannot be sterilized by boiling as heat converts the bicarbonate to toxic carbonate. The required volume of fresh double-distilled water should be sterilized and cooled. The chemically pure sodium bicarbonate is then added *in the cold* under aseptic precautions and the flask agitated gently until solution is effected.

4. Sodium citrate.

This is not decomposed by heat and can be sterilized in solution by boiling. Recent research suggests that it may replace the bicarbonate when alkaline solutions are required. The chemically pure salt should be used, and a sintered-glass funnel is to be preferred to the filter paper method of filtering solutions. A funnel of porosity 17G4 (45μ) is fine enough to give a water-bright solution free from any visible particles.

II. Medicinal Enemata.

Owing to the incidence of amoebic and chronic bacillary dysentery in tropical patients rectal injections such as yatren (quinoxyl) and bismuth subgallate in cod liver oil are frequently in demand.

1. Solutions of iodohydroxyquinolinesulphonic acid (chiniofonum, quinoxyl, yatren).

This light-yellow odourless powder consists of a mixture of approximately 4 parts of 7-iodo-8-hydroxyquinoline-5-sulphonic acid and 1 part of sodium bicarbonate, and contains 28·2 to 29·6 per cent. of I and 18·to 22 per cent. of $NaHCO_3$. It is stable in the tropics when kept dry and protected from light. For dispensing purposes it is convenient to prepare and stock a 5 per cent. solution. This can be diluted with distilled water to the strength required by the prescriber, the usual percentage being 2·5. *Tap water must not be used.* The vessels used for preparing and storing the solutions must not contain any trace of acid.

About four-fifths of the total volume of freshly-distilled water should be heated in a wide-mouthed vessel to a temperature of 60° to 80° C. (but *not above* 80° C.). The powder should be weighed out and added slowly, in small quantities and with continuous stirring, to the water. There will be effervescence with evolution of CO_2. When this is complete, the solution should be made up to the required volume with distilled water. Solutions must not be boiled as

this causes decomposition. Solutions so prepared will remain stable for some time but it is better to make them freshly every few days.

Dosage.—The course may consist of ten daily rectal injections of 200 c.c. (sometimes of 8 ounces) of a 2·5 per cent. solution (*i.e.*, 5 grammes of the powder in 200 c.c. ; 87·5 grains in 8 ounces) ; but strengths varying from 1·5 per cent. to 5 per cent. or increasing from 2·5 per cent. daily by 0·5 per cent. to a final strength of 5 per cent. have been used.

The solution should be run into the funnel at a temperature of 110° F. on administration to ensure the injection being given at body temperature.

2. COD LIVER OIL ENEMA.

This is usually prescribed as " Cod liver oil enema 1 in 4 " and is an oil in water emulsion. To make 80 ounces of this take 1 pint of the oil and make an emulsion *secundum artem* using 5 ounces of pulv. gum. acacia as the emulsifying agent. This is a rather expensive item but the results fully justify its use. On no account must such irritating emulsifying agents as tincture of quillaia be used, as they cause pain and distress to the patient. The emulsion so made is diluted with water to the required volume. It will keep for several days and without separation, though should this happen a homogeneous preparation is again produced by vigorously shaking the bottle.

Dosage.—4 to 8 ounces injected daily.

3. BISMUTH SUBGALLATE 5 PER CENT. IN OLIVE OIL OR COD LIVER OIL.

The important thing to remember about this preparation is that suspending or emulsifying agents must not be used. Grinding in a mortar or by mechanical means is the best method and it must be slow, gradual and thorough. If obtained from a wholesale chemical firm, it should be supplied in wide-mouthed bottles. The bismuth salt, being heavy, sinks to the bottom of the bottle. Mechanical stirring with a suitable rod is the only way to ensure an even distribution of the salt throughout the suspension. No amount of shaking or of standing the bottle in hot water will avail without stirring. This is especially important where the preparation is stored even in small bulk, to prevent the successive amounts removed being of different strengths. Ordinary dispensing glass rods are not usually sufficiently strong, thick or pointed for the purpose. The bottle must be well shaken after the contents have been stirred. Nurses should always be reminded of the necessity for this mechanical stirring.

Dosage.—A warmed injection of from 4 to 8 or even 10 ounces daily, per rectum.

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THE THIRTY-THIRD ANNUAL GENERAL MEETING

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Manson House, 26, Portland Place, London, W.,

on

Thursday, 20th June, 1940, at 4.30 p.m.

THE PRESIDENT

Sir S. RICKARD CHRISTOPHERS, *C.I.E.*, F.R.S., Colonel I.M.S. (retd.),

in the Chair.

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1940.

The **Hon. Secretary,** Dr. WENYON, presented the Thirty-third Annual Report, copies of which had been circulated.

Reference was made to the successful meetings held during the year and the regular publication of the TRANSACTIONS.

It was also noted that as was to be expected through the outbreak of war the number of Fellows on the Register was less than in the preceding year ; this decrease of fifty was due, not to resignations, but to a falling off in the number

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of new Fellows. The hope was expressed that every Fellow of the Society would do his best to encourage others to join so that the membership would be kept up and the Society enabled to weather the storm.

Dr. F. Hawking proposed the adoption of the Report. This was seconded by **Dr. G. H. Gallagher.**

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31st MARCH, 1940.

The **Hon. Treasurer,** Dr. OSWALD MARRIOTT, presented his Report with the accounts and Balance Sheet prepared by the Auditors, Messrs. W. B. Keen & Co.

He called attention to the inevitable decrease in letting the Hall ; and said that the tenancy of the maisonette was terminating at Christmas. On the other hand, it had been possible to effect certain reductions in Expenditure.

The amount of the debt on Manson House had been reduced by £655 during the year ; but it should be noted that the usual annual reserve for further repayment out of ordinary Income had not been made, as it was considered wiser to keep all balance available for general expenditure in case of necessity.

Mr. U. F. Richardson proposed the adoption of the Treasurer's Report. The resolution was seconded by **Dr. C. J. Hackett** and carried.

ELECTION OF AUDIT COMMITTEE.

The re-election of Dr. VINCENT HODSON, Dr. W. E. COOKE and Col. F. P. MACKIE as members of the Audit Committee was proposed by Sir WILLIAM WILLCOX, seconded by Col. C. H. BARBER, and carried unanimously.

This concluded the business of the Annual General Meeting.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 20th June, 1940, at 4.45 p.m.

(After the Annual General Meeting.)

THE PRESIDENT

Sir S. RICKARD CHRISTOPHERS, *C.I.E.*, F.R.S., Colonel I.M.S. (retd.),

in the Chair.

PAPER.

FIELD EXPERIENCES WITH THE SULPHANILAMIDE GROUP OF DRUGS IN THE SOUTHERN SUDAN.

BY

J. BRYANT, M.D., F.R.C.P.E.,

AND

H. D. FAIRMAN, M.B., B.S.

Sudan Medical Service, Khartoum, Sudan.

INTRODUCTION.

It is with a good deal of misgiving that I find myself here this evening in reply to an invitation to address this Society on some field experiences with the sulphanilamide group of drugs in the Southern Sudan.

The conditions under which most of these observations were made were often very primitive ; much of the treatment was in the hands of illiterate native dressers and case histories were sometimes difficult to take at secondhand.

The area served by the medical centre concerned is large and is fairly

heavily populated ; all too plentiful surgery at home, emergency calls from outside dispensaries for aid in·epidemics or fights or for other purposes make study impossible.

The medico becomes a " Jack of all trades and master of none," mails are infrequent and journals arrive weeks after publication.

The country has advantages, however, It is still largely the Africa of JUNKER and SCHWEINFURTH, of EMIN, GESSI, Sir SAMUEL BAKER and the ill-fated Miss TINNY. Many of the tribes are unspoilt and make excellent patients who give good and accurate histories.

The climate unfortunately is bad. White men do not thrive, malignant malaria and blackwater fever being amongst the chief evils.

I would ask you therefore not to be too critical of this very sketchy work.

The bibliography of this contribution may appear very incomplete, but the literature is so vast that although many papers and articles have been consulted only the few which directly concern any particular point in this evening's subject are quoted.

CHARACTERISTICS OF TRIBES IN THE BAHR EL GHAZAL.

Two very different anthropological groups are to be found in the old Bahr el Ghazal. They may roughly be divided into those possessing cattle, living in fly-free, flat, grassy, swampy country, park-land and light forest. The others live in tsetse country and are cattleless.

The former are the Dinka, people of great stature and great beauty, who cultivate the dry land in the rains and tend their immense herds in the grass-lands when the seasonal inundation abates. Proud, quarrelsome, argumentative, lazy, drunken and litigious, they are for all that very charming and amusing people and provide the bulk of the medical work in Wau.

The tribes living in the forest country differ greatly in appearance and mode of life from the great Nilotic tribes such as the Nuer and Dinka. They have a different outlook on life, have short instead of long heads, wear clothes, do not remove the lower incisors of children.

The Nilotic is primarily a pastoral spearman, the forest dweller a bowman-cum-spearman, agriculturalist-cum-hunter.

The " Forest Dwellers," with the exception of the Azande, suffered greatly at the hands of the slavers and are few in number. The Azande and the Dinka, however, were too much for the Arabs. Their countries were inaccessible, and what the Dinka conceded to the Zande in organization and tribal discipline he amply made up for in savagery.

Tribal Susceptibilities.

From the foregoing, as might be expected, tribal groups in the Southern Sudan react very differently to various drugs and diseases. Bismuth for yaws

can be administered in full doses to Nilotics (Dinka, Shilluk and Nuer), whereas caution must be excercised with the same drugs in forest dwellers (Azande, Bongo, and others) or a dangerous stomatitis results. Chloroform is ill tolerated by Nilotics, well by the Azande. Bone lesions in yaws are common in Nilotics, uncommon in forest-dwellers. Hookworm causes a profound and intractable anaemia in Dinkas, whereas the Azande are not affected to the same extent. It does not follow, therefore, that the results of treatment recorded here would have their counterpart in Europe or even in other parts of Africa.

I. CEREBROSPINAL FEVER.

Epidemic cerebrospinal fever in the Sudan has a very high mortality of between 65 and 85 per cent. of cases. An outbreak is regarded as a visitation from God, tribes become thoroughly upset and the various medicine men and witch doctors do a roaring trade in charms and prayers and their flocks and herds grow apace.

At the beginning of an epidemic in the Dinka country of Equatoria in 1939, we had treated a few isolated cases with prontosil by the mouth. None of these cases was in the acute stage and they had been ill for some time. Many of them recovered although the mortality was very high indeed in the untreated acute cases.

The following example of the high mortality is related. An urgent call came through to Wau saying that an important Chief needed help at once. He had thirty-seven cases of meningitis in his villages and had had them carried to the road and had placed them in grass shelters. One of us left at once with staff but some 40 miles from our destination the car became stuck in the mud. We were delayed 15 hours. On arrival thirty-three out of the thirty-seven were dead. A post was established. Two dressers and a British officer treated the next 250 cases with only two deaths using M. & B. 693 in saline suspension.

This figure may sound fantastic but is largely due to the fact that that particular section of Dinka lived along a sandy ridge and were easily reached. A native on a bicycle rode up and down the road at its far end treating the remote cases and the nearer ones were taken in by car or carried in at once. This meant that most of the cases were seen within an hour or two of the first symptoms and few had been ill more than 2 days. Local medicine men lent their aid free of charge and encouraged the relatives in their own picturesque way by spitting on the heads of the sick in benediction and pouring beer and dust or flour on the relatives. Sacrifices of goats were allowed behind the quarantine.

SULPHANILAMIDE-P.

At the commencement of the outbreak a sample of 100 tablets of sulphanilamide-P were sent by British Drug Houses to one of us (H. D. F.). The

instructions said that one tablet should be dissolved in 40 c.c. of saline at 37° C· and ingested at that temperature. It was found that this was too bulky a quantity to use in field work, that on cooling the drug precipitated and on boiling turned in solution a dusky red.

. The results, however, of the injection of the precipitate were extra-ordinary and we only lost one out of the twenty-one cases treated with intra-muscular and intrathecal injection combined with prontosil by the mouth. The doses were very small and many of the cases appeared to be beyond hope. As an instance of the small doses administered the following case of a moribund boy of 6 is quoted at random.

He had been taken ill five days previously, was *in extremis* with broncho-pneumonia and cerebrospinal meningitis. He was unconscious, the eyes were fixed and open and the pupils were dilated. In 4 days' time he returned home after a total of 1 gramme of sulphanilamide (0·5 grammes in the buttock and 0·5 grammes in the theca) and 1·5 gramme prontosil by the mouth.

It was impossible to use more sulphanilamide at this time as the remainder of our small stock was lost during sterilization.

It was noticed, however, that where lumbar puncture had been refused by relatives the patients although improved were not nearly as well as those who had had puncture, and that where puncture was subsequently performed it was found that the cerebrospinal fluid had turned the peculiar dusky red of the sulphanilamide injected in the buttock some 12 to 15 hours previously. This assured us that the drug was rapidly excreted into the theca in spite of inflamma-tion and increased tension.

FIRST CASES TREATED WITH M. & B. 693.

When our first supplies of M. & B. 693 arrived we attempted to treat cerebrospinal meningitis by the oral route using the same dosage as for pneu-monia. Our results were poor. The big and powerful natives were difficult enough to handle and forcible feeding was well-nigh impossible. They fought and bit and spat the powdered tablets out. When big doses were swallowed by the very sick they invariably died, the course of the disease was too rapid and the action of the drug too slow.

On the assumption that suspensions of imperfectly dissolved sulphanilamide were administered without ill effects we powdered up a few tablets of M. & B. 693 and injected them after sterilization in the quantity of one tablet suspended in 10 c.c. of saline.

This was admittedly a shot in the dark and was in the nature of a desperate remedy. The results were miraculous and after trying the intrathecal route for some time we decided that it had no advantages over the intramuscular and have urged this latter route exclusively with suspensions ever since.

Dosage.

A table of the first 160 successfully treated cases of a series of 168 taken from the *Lancet* (BRYANT and FAIRMAN, 1939) is given here.

Age group.	Type of attack.	Number of cases.	Average total dosage.
Infants and children under 5	Mild	15	0·5 grammes
	Severe and fulminating	40	1·25 „
Children aged 5 to 14 ...	Mild	15	1·25 „
	Severe and fulminating	50	2·0 „
Adults	Mild	6	2·25 „
	Severe and fulminating	34	3·25 „

Since these first few cases were treated the dose has been increased. The average severely ill adult now receives ten tablets instead of 3·25 grammes or just over six tablets.

M. & B. 693 in Oily Suspension.

Whilst we were treating the first cases with saline suspensions, ampules containing M. & B. 693 in oily suspension were received. This preparation for field work was most unsatisfactory. The drug was extremely difficult to extract, blocked needles, and did not mix with the cerebrospinal fluid. Droplets of oily suspension were removed at lumbar puncture 48 hours after injection, apparently unchanged.

Preparation of Saline Suspensions.

At first finely powdered tablets of M. & B. 693 were suspended in normal saline in a strength of one tablet to 10 c.c. Later it was found that two tablets in 10 c.c. were as easily given. At present, 100 tablets are made up with 500 c.c. of saline and sterilized by boiling for an hour in a water bath. For field work, this stock suspension is poured into 100 c.c. bottles which are sealed with a rubber cap.

If sterilized in this way a very fine flocculent suspension is the result. This does not block serum needles.

MORTALITY OF TREATED CASES.

At the end of the 1939 epidemic the mortality of treated cases was in the region of 5 per cent. Our friend, Dr. USHER SOMERS (1939) working at Rumbek some 150 to 200 miles to the east, recorded a 10 per cent. mortality using rather different methods with smaller doses in higher dilutions.

Dr. FAIRMAN reports a rise in the mortality rate in the Wau area to 10 per cent., an increase of 5 per cent. over last year's figures. The type of disease encountered seems to have altered somewhat and most of the fatal cases do not appear to react to treatment and many cases have needed often repeated lumbar puncture, whilst in the 1939 outbreak patients were seldom drained more than twice.

Types of Cerebrospinal Fever Encountered.

The severe and fulminating form of the disease was much commoner than the milder form which becomes chronic and not infrequently ends in recovery. Death within 12 hours was not uncommon.

The most dangerous type was the septicaemic, often because the disease was not recognized. The picture of a cold, collapsed child with a rapid pulse, very little neck rigidity and clear spinal fluid does not look like cerebrospinal fever. If the child improved, subsequent lumbar puncture produced the typical yellow fluid found after injection of M. & B. 693, slightly turbid and containing pus cells and a few meningococci. In all of the few blood slides of ordinary severe cerebrospinal fever examined, meningococci were found.

Arthritis, iritis, corneal ulcer and various flaccid palsies and retention of urine were all seen. Early deafness did not seem to be relieved by treatment, whereas paralized limbs tended to recover later.

Other Forms of Meningitis.

Of several cases of pneumococcal meningitis treated not one recovered.

A streptococcal meningitis following operation on an extensive middle meningeal haemorrhage cleared up on sulphanilamide-P injections. The patient, however, developed a cerebral abscess which was located and drained but was not affected by further sulphanilamide therapy. The patient died 2 months after admission.

A peculiar and fatal form of meningism with clear fluid which is not uncommon in children after influenza was seen during the beginning of the 1940 epidemic. These cases were treated on similar lines to cerebrospinal meningitis and all recovered ; but recovery was much slower than from cerebrospinal meningitis although the cases were not nearly as ill at the outset.

Bacteriology.

Bacteriological examinations were naturally impossible under these conditions, but it must be realized that the strain of organism found during an epidemic will have a very marked effect on the mortality rate.

Thus, although with resistant and virulent strains of meningococci one might expect a 10 to 20 per cent. mortality, with the culturally different slightly drought-resisting *crassus* form at present being isolated in Egypt one might find a mortality as low as 1 per cent.

Lumbar Puncture.

Contrary to European practice drainage of the theca was always in the sitting instead of the prone position for the following reasons :—

1. To the unskilled native dresser it is far easier to get the correct line and position than when the patient is recumbent.

2. Drainage of thick, purulent fluid is possible in the erect, impossible in the prone, position.

3. Complete drainage is possible.

Note.—It was noticed that at subsequent lumbar puncture of cases where an intrathecal injection of M. & B. 693 had been given, a jelly-like semi-transparent cast could be removed from the lumen of the needle after drainage. This rather puzzled us at first until one of us (H. D. F.) happened to place this jelly on an iodine swab and found it turned deep blue. It is presumed that this was starch and possibly has some bearing on the efficacy of the treatment of gonorrhoea by producing a febrile reaction.

SUMMARY.

A very incomplete description has been given of the methods first used in treating epidemic cerebrospinal fever in the field in the Southern Sudan.

No reference to solutions is made for supplies of these preparations were limited.

From a wide experience of the severe and fulminating form of the disease, it was found that oral administration seldom saved a case, was wasteful and difficult to give. Severe cases should *always* be treated by injection. Oral administration may be used during recovery only.

Lumbar puncture should always be performed and drainage should be as complete as possible. If a big injection of M. & B. 693 is given at the outset, a repetition of drainage is needed only in about 30 per cent. of cases.

Rate of recovery seems to be directly proportional to the size of the initial dose and the speed with which the patient is treated after developing symptoms.

Doubtful cases should be treated at once as cerebrospinal fever and a diagnosis made later.

This work was done solely amongst the Dinka tribe and it is not claimed that similar dramatic results would be obtained elsewhere.

No reference is made to contemporary literature as, it is intended that the foregoing should be an account of personal field experiences only, not a scientific discussion.

II. GONORRHOEA.

Before relating these experiences it would be as well to say a word or two about the town of Wau where this work was done.

Wau until recently was the capital of a province called the Bahr el Ghazal.

Its history had been an evil one until that great Italian GESSI smashed the slavers and ended for a while the curse of Arab misrule with the execution of Suleman, the son of the notorious Zubeir.

After GESSI's political eclipse and transfer, slave dealing again became rampant, and was in full swing when the intrepid MARCHAND arrived with his handful of men who had carried their iron boats in sections from the Atlantic.

At Wau MARCHAND tarried awile, built Fort Desaix and it was from Wau that he set out for far away Fashoda on the White Nile.

Then came the reconquest of the Anglo-Egyptian Sudan, the reoccupation of the Bahr el Ghazal and the gradual pacification of the country. The Egyptian battalions gave way to Sudanese regiments who in turn handed the garrison duties on to the Equatorial Corps of the Sudan Defence force.

It can be well imagined that the years of slavery had reduced to ragged, beaten and hopeless remnants the once large and intelligent tribes that inhabited the well-watered land of plenty west of Wau to the present boundary of French Equatorial Africa.

The best and bravest of these peoples had been sold in the markets of Cairo, Khartoum, and the Hedjaz, and it is little wonder that the remainder, many of whom drifted to Wau, became totally detribalised, losing all sense of tribal loyalty, lacking all background.

Here the vices of the Oriental, mated with those of the neo-animist, begot that truly unlovely child—the native town of Wau.

This digression is necessary if the conditions of life in Wau are to be understood, for hashish addiction, drink and prostitution were the pastimes of the inhabitants and the effect on garrisons of police and troops can well be imagined.

GONORRHOEA AMONGST TROOPS AND GOVERNMENT SERVANTS.

Investigation of returns showed that for some time past 40 per cent. of the troops had been detained in hospital per year for venereal disease. The number of days lost was serious and with the gathering of the war clouds over Europe it was decided that this state of affairs must cease.

Concern was also felt at the spread of veneral disease amongst the pastoral Dinka and sterility amongst their women.

The officers of the Sudan Defence Force, the political officers in Wau, and medical services foregathered and decided on a campaign along the following lines :—

1. All motor drivers, civilian, Army or Government, to be subjected to periodic, unexpected, inspections.

2. All known prostitutes and unattached women to be inspected, and if uninfected returned to their tribal units and refused re-entry into Wau. All those infected to undergo treatment.

3. Frequent surprise inspections of troops and police after early morning physical training. The wife of any infected man automatically entering hospital for treatment.

4. Insistence that the names of the person or persons suspected of transmitting the disease be revealed. These persons to be produced by the political officers for examination and, if necessary, treatment.

5. Concealment of venereal disease to be a punishable offence in the Army.

6. If a person infects another with venereal disease, being cognisant of the fact that he or she has the disease, it shall be regarded as equivalent to causing hurt and compensation shall be granted to the injured party.

Now all this sounds very high-handed, much dragooning of the natives was necessary, and it is well-known that compulsion and punitive measures may be a two-edged sword when applied to venereal disease. Our pessimism over the campaign was proved to be unfounded and results were soon forthcoming.

In February, 1939, the venereal disease rate amongst the " bachelor women " of Wau was 93 per cent. ; in November it was 11 per cent.

Again, to show that no malice was borne from our taking the strong line, the following experience is related.

It must be remembered that for over a year the women of Wau had been subjected to numerous surprise round-ups, compulsory examinations and treatment ; and had been very considerably harried.

In mid-February of 1940, it so happened that one of us (J. B.) had to be evacuated by 'plane suffering from septicaemia, in a very serious condition indeed.

We shall never forget the sight of the " bachelor women " in Wau crowding round the 'plane, dressed in their best, the tears streaming down their faces ; or their wildly waving arms as their shrill trilling was drowned by the roar of the engines as we taxied off.

Differences in Results obtained in England and in the Sudan.

It was fortunate that in Wau the military and police garrisons were kept in the same place, enabling us to check and countercheck our results and call for re-examinations when we had the time to perform them.

As will be seen, our results vary greatly from those obtained by highly skilled observers of vast experience in this country. It was on account of these very differences that a good deal of care was taken to ensure that these findings are as accurate as possible, and that they are not due to the first rosy flush of success or wishful thinking.

Firstly, the dosage has been small and the relapse rate low. It is unfortunate that the pressure of surgical work and epidemic cerebrospinal fever prevented us from being able to complete our analysis of some 400 observed cases. Some of these were treated with sulphanilamide, some with sulphapyridine and some with both.

reaction that follows injection. Is the starch or starch-like substance, contained in the tablets responsible for this reaction ? Does the resultant febrile reaction take the place of that produced by protein shock or vaccine ? Does M. & B. 693 in powder form produce the same reactions as the powdered tablets ? Is this reaction responsible for the results obtained with such small quantities of drugs ? (See typical temperature charts on page 127).

Differences of Southern Sudanese and European Gonorrhoea.

All the complications of gonorrhoea seen in Europe are to be found in the Southern Sudan and all react to the sulphonamides. It is worthy of note, however, that although prostatic involvement is the rule in chronic and sub-acute cases, prostatic abscess is rare, whilst stricture is equally uncommon. This latter observation is interesting as natives are frequently becoming reinfected. Perhaps we are dealing with a weakly race of microbe or a resistant population. The latter supposition seems most unlikely.

Reasons for Believing Relapse Uncommon.

Although unable to produce a carefully collected and minutely examined series, there are certain facts that go to uphold the belief that the small quantities of drugs that have been used have produced lasting cure.

1. Women discharged as cured have since become pregnant after having been considered barren before and have remained free of discharge.

2. JANET maintained that the best culture media for the gonococci was the uninfected male urethra. I have yet to see amongst the police and military a man reinfected, or infected by his wife, who has been discharged from hospital as cured.

3. Relapses are very rarely seen at surprise inspections of troops and police. These inspections are frequent and a discharge, however slight, is seldom missed. Reinfection is nearly always admitted.

4. In the spring of 1939, of a marching-out strength of 145 troops thirty-three fell out in the first 2 days of training for relapse of gonorrhoea. In 1940, of 165 men not one fell out for venereal disease during an arduous training march of 700 miles at a rate of 4 m.p.h. in full war equipment. They averaged 19·5 miles a day for the whole march, and as a free day occurred occasionally they covered 40 miles and more a day on several occasions. Surprise inspections were carried out during manoeuvres and not a single man had a discharge.

I am obliged to BIMBASHI N. BOYER, Commandant of Troops, Wau, for his co-operation in this campaign and for this report.

As has already been stated, 40 per cent. per year of these troops have in the past been admitted to hospital for venereal disease.

These few incomplete observations are given for what they may be worth, in the hope that others in more favourable circumstances may be able to follow them up.

Intramuscular Injections of Powdered Tablets of M. & B. 693 in Saline.
Advantages.

1. Suspensions are easily prepared and keep.
2. The dose is very much smaller and therefore cheaper than by the oral route.
3. Its action is very quick.
4. Toxic symptoms—at least in Sudanese—and abscess formation are very rare.
5. Suspensions appear to be safe in the hands of native dressers.

Disadvantages.

1. Injections of suspensions are painful. The discomfort complained of by a Dinka might be agony to a European.
2. Owing to the marked febrile reaction, patients should be treated in hospital if possible.

III.—Tetanus.

We come now to the use of sodium evipan in conjunction with M. & B. 693 under field conditions for the treatment of tetanus. The mortality rate in twenty-two cases was 22·7 per cent.

During an epidemic of cerebrospinal fever in 1939 a number of cases of tetanus were brought to Sudan Medical Service dressers for lumbar puncture and injection with M. & B. suspensions.

Now the Dinka know tetanus well by repute. They call it (not inaptly) lower incisors, and consider it so fatal that medical help is of no avail. The mortality rate in tetanus must be very high in untreated cases—probably in the region of 80 per cent.

During the constant patrolling of the epidemic areas we saw some twenty-two cases of tetanus in 6 months. These were treated with sodium evipan and M. & B. 693, mainly in treatment centres in the bush. The headache, stiff neck and back of cerebrospinal meningitis had been confused with the opisthotonos of tetanus, and tetanus came to be regarded as a kind of cerebrospinal meningitis.

So anxious are many natives to reach a cerebrospinal-meningitis treatment centre as soon as possible after the onset of headache and stiff neck that some of these cases of tetanus were seen very early on, before convulsions had commenced. Diagnosis was only possible when the first unmistakable classical signs and symptoms of the disease became evident.

Those who have served amongst the Nilotic tribes of the Nile basin must often have wondered at the rarity of tetanus. The hundreds of stab wounds, game injuries, maulings from the great cats with the terrible attendant sepsis, bone damage and gangrene are ideal for the development of the disease. We

see, as a matter of fact, very little tetanus indeed, a case or two a year, sometimes none.

A wounded man, as often as not, is led or carried in by friends and relatives with his wounds dressed with cow dung compresses over which pieces of filthy rag or leather are tightly tied.

Cow Dung and *Bacillus tetani.*—TOPLEY and WILSON quote various workers' observations on *B. tetani* in cow dung.

TOLEDO and VEILLON (1891) demonstrated its presence in cow dung, whilst NOBLE (1915) failed to isolate it in twenty-one specimens, and KERRIN (1929) found *B. tetani* in four out of twenty-one samples.

Conversations with friends and colleagues who are bacteriologists lead one to believe that *B. tetani* is a common inhabitant of the gut of cattle.

How comes it then that tetanus is so seldom seen ? From experiences last year it seems that tetanus is not at all uncommon but it is being seen now because the natives believe it a curable disease akin to meningitis.

SUMMARY OF CASES OF TETANUS TREATED.

Lesion.	Number of cases.	Number of deaths.
Guinea worm	6	1
Strangulated hernia (resection of gut) ...	1	—
Lion bite	2	—
Burns (epileptic)	1	—
No visible injury	8	3
Motor accident (compound fracture of leg)	1	—
Thorn in foot	1	—
Accidental spear wound of foot	1	—
Spear wound of lung	1	1
Total ...	22	5

IDIOPATHIC TETANUS.

In this series eight cases showed no visible injury, but Africa is a country where injuries from fish spines and fish spears, thorns, and grass are of everyday occurrence and pass unnoticed.

In regard to the development of tetanus in wounds it must be remembered that TULLOCH (1919) found *B. tetani* nineteen times in a series of 100 war wounds from which no tetanus developed. Some other factor seems to be necessary for the development of the disease. Thus BULLOCK and CRAMER (1919) demonstrated that certain ionizable calcium salts, if injected with toxin-free spores of

tetanus, led to its development. It is possible, therefore, that some essential factor for the development of tetanus is missing in the Bahr el Ghazal.

GUINEA WORM AND TETANUS.

It will be seen from the Summary of the Treated Cases that there are six cases of guinea worm infection. In many years' service in the Bahr el Ghazal, where one of necessity acquires a wide experience of dracontiasis, this is, to the best of my belief, the first time that tetanus has been seen following Guinea worm. It may be that this common complaint was incidental and not causative. Guinea worm is so common that had tetanus been a recognized sequel it is unlikely that it would not have been better known. On the other hand, what lesion is more suited to the development of tetanus than the long, tortuous, infected channel left by the worm?

TWO TYPICAL CASES OF TETANUS IN CHILDREN.

Recorded by Dr. DOUGLAS FAIRMAN.

Case 1.

8.12.39.—Dinka girl, *aet*. 3, brought to rest house in evening, said to be suffering from cerebrospinal meningitis. Headache since previous evening. Temperature 100° F., slight neck rigidity. No spasms. Kernig's sign? L.P. fluid clear. Pressure +. 1 gramme M. & B. 693 in saline suspension intramuscularly. Diagnosis? Relieved by lumbar puncture and slept well.

9.12.39.—Spasms commenced. Parents recognizing tetanus took child away to die.

10.12.39.—Child brought back by police in early morning. Almost continuous spasms with trismus. No visible injury or history of same. Typical carpo-pedal spasms absent. Evipan 0·5 gramme intravenously—later a further 0·25 gramme and 1 gramme M. & B. in buttock. Glucose saline per rectum.

6 p.m.—Evipan 0·5 gramme to ensure a quiet night although still anaesthetized.

11.12.39.—Awoke from evipan. Still had spasms. 0·5 gramme evipan repeated.

5 p.m.—Awoke. No spasms.

12.12.39.—No spasms.

13.12.39.—Still no spasms but trismus remains. Can drink with comfort. Well on road to recovery. Treatment discontinued.

Case 2.

Dinka girl, *aet*. 8, brought to dispensary as case of cerebrospinal meningitis.

4.30 p.m.—Dresser recognized tetanus and fetched one of us (J. B.) who happened to be on trek and staying at treatment centre.

6.11.39.—Very sick child in almost continuous spasms of tetanus. Ill for 2 days—home 20 miles away, 1 day on road. Thorn in foot 14 days previously. No visible injury. 0·75 gramme evipan intravenously. L.P. 45 c.c. clear fluid under pressure. 1·5 gramme M. & B. intramuscularly.

9 p.m.—Morphia ⅛ grain.

7.11.39.—General condition better but teeth tightly clenched. Dinka " dentist " called in to remove lower incisors at parents' request.

(*Note*.—Nearly all Nilotic tribes, Nuer, Dinka, Shilluk, etc., as a ritual custom remove with a fish spear the lower incisors when a child is from 9 to 10 years old.)

0·5 gramme evipan intravenously.

6 oz. hot tea full of sugar administered without choking by nose *via* catheter instead of services of " dentist ".

5 p.m.—Seems generally worse but can half-open mouth. Can push in soft food and swallow slowly yet completely, spasms returned after food. Looks very ill. 0·5 gramme evipan and 1 gramme M. & B. intramuscularly.

8.11.39.—Much improved. Can half-open mouth and swallow more easily. Spasms still present but less severe. Evipan 0·5 gramme.

9.11.39.—Well on road to recovery.

12.11.39.—Still painful intercostal spasms and intermittent spasms of platysma. Fixed "*risus sardonicus.*" Walking slowly with staff with inturned toes. Very stiff generally.

15.11.39.—Discharged. Still very stiff. No spasms.

Note.—Although both these cases made such rapid recoveries they would both remain very stiff for a long time. The set face and the partial spasm of the muscles of the shoulder girdle and trapezius take many days to pass off.

Tetanus and Mortality in Children.—In regard to these two cases the following quotation from Price's textbook of medicine is significant : " . . . Children and infants seldom recover."

Points of Interest in Individual Cases.

One Dinka man was admitted suffering from fulminating cerebrospinal meningitis. He regained consciousness and made an uneventful recovery after receiving 2·5 grammes of M. & B. in suspension.

The dresser in charge of the quarantine in Wau reported that the man might have relapsed but looked as if he had tetanus. His spinal fluid—extracted under anaesthesia—was much improved but still turbid and was the typical yellow colour one sees after treatment with this drug. He had a small septic wound in the scrotum from which a guinea worm had been extracted with a spear some 14 days previously.

This patient was treated with continuous and then intermittent evipan narcosis and was given 3 grammes of M. & B. 693. Convalescence was very slow and it is interesting that the original 2·5 grammes of sulphapyridine did not abort the disease.

The man who had a resection of gut undoubtedly acquired the disease at the operation.

The two brothers mauled by the same lion before they killed it were badly scratched and bitten. Both had the ilium on one side bitten through and smashed. In spite of the cauterization of wounds after excision and the employment of dipterous maggots in one case they both developed tetanus 4 days after being injured. Both recovered. Several operations for removal of sequestra were performed after their recovery from tetanus. They were treated after lumbar puncture by continuous and then intermittent evipan narcosis and by injections of M. & B. 693 (2·5 grammes, 2 grammes and 2 grammes).

FATAL CASES.

The man suffering from the spear wound in the lung had little hope of recovery. He died of gangrene of the lung and tetanus 8 days after admission.

Two girls did not react to treatment. Another developed laryngeal spasms when apparently well on the way to recovery after 6 days of treatment. She was dead by the time we reached the ward.

One Dinka man died after one injection of evipan and some M. & B. He had received the last available ampoule of evipan. Torrential rain closed the road and the car taking drugs and dressings to the treatment centre could not proceed. The unfortunate man died 4 days later. He had been seen personally and it is possible he would have lived had evipan been available.

DOSAGE AND DRUGS.

Evipan is given to produce continuous drowsiness and the dose regulated to control convulsions. Muscular rest has been aimed at. Narcosis can be prolonged by morphia and other sedatives. In dispensaries 1 gramme has been given to adults in the morning and the evening as supplies of drugs are limited. If necessary 1 gramme is given in the afternoon also. Drip administration is impossible in the bush.

Tetanus and gross sepsis have been treated with three to five intramuscular injections of 1·5 to 2·5 grammes of M. & B. 693 in saline suspension.

Treatment of Tetanus with Sodium Evipan Alone.—Since the introduction of sodium evipan this wonderful drug has been used for the few cases of tetanus that were encountered from time to time. Results have been fair and unexpected recoveries made. From a purely humanitarian view, of course, it has been a godsend.

Using this anaesthetic alone, recovery has always been very slow and wasting pronounced. Injections of magnesium sulphate have not been used, but lumbar puncture has been observed to bring some relief,,the fluid being as a rule under considerable pressure.

Feeding under Anaesthesia.—Nasal feeding under semi-anaesthesia is not difficult. The swallowing reflexes are not abolished, the patient swallows quite well and chokes very little. The rough and ready methods of bush medicine enabled us to feed, and feed adequately, large and powerful patients and small children with milk and soups, gruel and tea and sugar.

DYCE SHARP (1939) noticed the relaxation of the jaw which followed large doses of sulphonamide. This interesting fact has been noted in the Sudan also.

COMMENTARY.

Of twenty-two cases of tetanus treated in the Southern Sudan, five, or 22·7 per cent., died. This is low mortality.

It is considered that these results cannot be explained entirely by the muscular rest produced by evipan narcosis, for recovery after treatment with M. & B. 693 combined with evipan is very much more rapid than when evipan is used alone.

The series is small and we may have been lucky.

Should the conclusions drawn from the foregoing cases be justified, the importance in war of these methods needs no comment. They are especially indicated where high temperatures and remoteness precludes the use of serum.

The Action of the Drugs under Discussion.—The action—as far as we can tell—of evipan needs no explanation, for if patients can be kept alive for a week on continuous narcosis and muscular rest alone, recovery is to be hoped for.

How does sulphapyridine act ? On the bacilli producing the toxin or on the toxin itself ?

Findings of Other Workers.

DYCE SHARP working on the Gold Coast had five consecutive recoveries using prontosil and other similar preparations which were given every few hours until the spasm of the jaw relaxed. If the patient could not swallow, injections in a gum suspension were made. Details of treatment are not given. The recorded mortality of tetanus in the Gold Coast is given as 75 per cent. but the author believes it nearer 80 per cent.

MAYER, in a paper read at the Académie de Médicine de Paris, states that of thirty control mice only one, or 3·5 per cent., lived after the injection of earth containing tetanus bacilli, whereas of ninety-five mice which were given one of these drugs (sulphanilamide, etc.), forty-one, or 43 per cent., survived.

We know that if huge doses of antitoxin are circulating in an animal, quantities so large that many other animals are capable of being immunized by its serum, that antitoxin will not save the animal if toxin be injected into the substance of the central nervous system.

Does M. & B. 693 in some way enable antitoxin to reach toxin in nervous tissue ? Why should the spasm of the masseters relax ?

One can well understand the bactericidal action of M. & B. 693 on the bacillus of tetanus, and possibly its destruction before its toxin produced symptoms, but no attempt is made to explain what we believe is its therapeutic action in developed cases of tetanus.

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DISCUSSION.

Sir William Willcox : I should like to congratulate the authors of this very interesting paper because it expresses the work of men doing their best when faced with great difficulties, and it must have been a great satisfaction to them when their efforts met with such striking success.

DR. BRYANT was confronted with a serious epidemic of cerebrospinal meningitis occurring amongst the native population of which he was in medical charge. He was well acquainted with the recently discovered great therapeutic value of the sulphanilamide compounds but of these he had only a very limited supply of one, *viz.*, M. & B. 693, the pyridine derivative, which was in the form of $\frac{1}{2}$ gramme tablets, a small quantity of starch being present to give the necessary adhesive property to press the powdered preparation into a satisfactory firm tablet. Dr. BRYANT and his colleague had not sufficient tablets for the usual oral doses, and there would have been difficulty in swallowing them in some of the severe cases.

They conceived the idea of boiling the tablets with saline for an hour and a half when a mucilaginous suspension of the drug was obtained so that doses of two tablets (1 gramme) of the drug could be administered in 10 c.c. of liquid intramuscularly.

They gave in this way, for gonorrhoea, daily doses of 1 gramme of M. & B. 693 intramuscularly for 4 or 5 days, then a break of 4 days, which was followed by a further similar course and so on as required. Dr. BRYANT obtained a remarkably high percentage of recoveries, as is shown by his statistics, and the favourable results quite equalled those obtained with doses of the drug five or more times as great when given in the usual manner by the mouth.

To what can this surprising result be attributed ? In my opinion the probable explanation is that the small amount of starch in the tablets used produced when given intramuscularly a certain amount of " protein shock."

The temperature charts showed a rise of temperature of about .2–4° after each injection which was consistent with this explanation.

It is well known that foreign substances, such as milk, peptone, typhoid vaccine etc., will cause a pyrexial reaction when injected hypodermically. It is also known that this reaction has often remarkable curative properties as, for example, in cases of arthritis and general infections.

It is reasonable to attribute the enhanced curative action of the M. & B. 693 to the combination of the therapeutic action of the drug with the " protein shock " caused by the other foreign substance (starch) present in the injected liquid.

Another factor in the success of Dr. BRYANT's treatment for meningitis was the fact that administration of the drug was begun at the earliest possible

moment, even before bacteriological confirmation of the diagnosis, and also that the drug was given intramuscularly so that quick and complete absorption into the blood stream was ensured.

The administration in courses of daily injections for 5 days repeated after a break of a few days is to be commended since it combines maximum efficiency with the removal of the danger of toxic effects from the drug.

I believe that success in the use of the sulphanilamide preparations is best attained by giving full doses for about 5 days so that a high percentage of the drug in the blood is attained, then a break of a few days to obviate the danger of toxic effects and afterwards repetition of the courses as is found necessary. We have all admired the ingenuity with which Dr. BRYANT and DR. FAIRMAN successfully combated the serious epidemic of cerebrospinal meningitis under great difficulties.

It is work of this kind, far away and under great difficulties, which makes tropical medicine a fascinating and romantic lifework.

Dr. S. Strahan : I should like to ask if the injections in the case of gonorrhoea and tetanus were given intrathecally as in the case of meningitis.

Dr. Bryant : We used to begin with intramuscular and intrathecal injections for meningitis, but very soon found that the intrathecal had no advantage over the intramuscular.

Dr. F. Murgatroyd : The remarkable results described by Dr. BRYANT in this interesting paper are as stimulating as they are surprising. In the case of the gonococcal infections I wonder whether the temperatures developed by the patients after the injections of the tablets might not have had a direct influence on the therapeutic results. Although strains of gonococci differ in their resistance to heat many strains succumb to temperatures tolerable to man, and there are numerous reports, particularly from America, of the treatment of gonococcal infections by the maintenance of patients at elevated temperatures for a number of hours, as for example by means of the Kettering hypertherm.

The President : Do they get marked success ?

Dr. Murgatroyd : Successes are recorded in as many as 90 per cent. of cases with a single session of 10 hours or repeated sessions of shorter periods at a temperature of between 106° F. and 107° F. It must be admitted that this temperature is relatively very high, but BALLENGER and his colleagues* suggest that lower temperatures, especially when combined with chemotherapy, may be equally effective ; they employ 3-hourly periods at 103-4° F. every other day

*BALLENGER, E. G., ELDER, O. F., & McDONALD, H. P. (1937). *J. Amer. med. Ass.,* **109,** 1037.

for three sessions combined with the administration of 4-5 grammes of sulphanil-amide daily. Although Dr. BRYANT's temperatures may have been somewhat lower they were repeated daily for 4 days and it would be interesting to examine in more detail their precise degree and duration. Another interesting matter bearing on the mechanism of Dr. BRYANT'S cures is whether with ordinary methods of administering sulphapyridine similar results could be obtained by injections of the excipient of the tablets. Incidentally, if the effect is due to injection of starch the term "protein shock" would require qualification. Whether the prolonged heating of the tablets in the preparation of the suspensions produced any change in the drug is another matter for enquiry and there is no doubt that the astonishing results achieved by Dr. BRYANT and Dr. FAIRMAN will stimulate further valuable research.

Wing-Commander G. L. M. McElligot: As my name is mentioned in the paper I would like to say one or two things. First of all, may I make a suggestion as to the success of what seems to be entirely inadequate treatment for gonorrhoea in this country. Is it not possible that what the last speaker said might be carried much further? The patient whom Dr. BRYANT partially cured of his gonorrhoea with an inadequate dose was later in the period of observation totally cured by an attack of malaria. Is or is there not very much malaria in his locality?

Dr. Bryant: It is extremely malarious.

Wing-Commander McElligot: The other point was that Dr. BRYANT seems to have had a remarkably low incidence of defaulters, who, of course, in civilian clinic practice are a bugbear. We at home cannot estimate our relapses thoroughly because we do not know that they will return to us. In addition to the relapses we know of, something between 12 and 27 per cent., following treatment with M. & B. 693, there may be still a larger number of whom we have no knowledge.

With regard to the oily suspension of M. & B. 693 : some 18 months ago I had the opportunity of trying it against an oral administration, and we found that its therapeutic effect was far below that of the oral product. But recently I have had the opportunity of treating cerebrospinal fever with Dagenan sodium, that is, the soluble sodium salt of sulphapyridine : the results have been remarkable and, as one would expect, there is a corresponding increase of the blood concentration of the soluble product as against the suspension.

Dr. BRYANT, when talking about treatment of gonorrhoea, did not give the proportion of his cases which did not react at all to treatment. We found with sulphanilamide that the proportion was comparatively high, there were something like 20 per cent. who did not react immediately ; but with M. & B. 693 our

proportion was low. We got the relapses later, and I am still convinced that they were true relapses. The men were subjected to a cross-examination and anybody who gave the faintest indication that he might have run another risk was put off the list. People in this country do not like to admit to reinfection, whereas the native of the Southern Sudan would presumably not be so particular about that sort of thing.

Dr. Bryant: As to defaulters. There was no chance of the police or military defaulting.

I think your experience with the oily suspension was the same as ours. We found it a most unsatisfactory drug. It was very difficult to handle and there was no comparison between results obtained by the oily and the saline suspensions.

As to relapses, I cannot give definite figures, but I must say that we made out that about 70 per cent. were free about 3 months after one course of treatment. We watched them very carefully.

COMMUNICATIONS.

MALIGNANT MALARIA AMONG DRUG ADDICTS.

EPIDEMIOLOGICAL, CLINICAL AND LABORATORY STUDIES.*

BY

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CONTENTS.

The object of this paper is to present a detailed study of malignant malaria, especially of the cerebral type, as it occurs among drug addicts who practise the common use of hypodermic syringes; and to present epidemiological, clinical, and pathological features of particular interest in this disease.

Malaria is generally considered an exotic disease by the lay or even medical inhabitants of non-endemic areas. Its occurrence at present in urban communities outside the tropics or southern climates is little stressed. In fact, except for therapeutic malaria induced in the treatment of general paresis or an occasional instance of its accidental transmission following blood transfusion, the disease has come to be regarded by many as a curiosity in metropolitan medical experience outside the tropics or endemic areas in the Southern United States.

HELPERN (1934) reported an epidemic of malaria confined to the drug addict population of New York City. He cited 49 cases all following common use of hypodermic syringes. This outbreak in New York, in which 21 individuals died, occurred within a year of the first report in this country of malaria among drug addicts using common syringes. In a short time sporadic outbreaks were recorded in various parts of the United States including the Pacific Coast, the South and the East, so that by 1934 we have the serious epidemic described by HELPERN in New York City.

Individual and isolated instances of this disease, and even an epidemic such as reported in New York if self-terminating, may well be regarded as medical curiosities. However, the continued appearance of new cases during the past five years in New York and elsewhere, and evidence that the aetiological parasite can be returned to its natural cycle in the mosquito, carries serious epidemiological implications. The Office of the Chief Medical Examiner (HELPERN) of New York has performed well over 100 autopsies on fatal cases of cerebral malaria in drug addicts. This number is especially significant of the probable number of infected individuals at large because of the present earlier recognition and treatment of the disease, with its attendant lower mortality rate than that experienced early in the series.

Since HELPERN's report we have had the opportunity of studying at some length the course of the disease in more than 200 drug addicts who contracted malaria following common use of a hypodermic syringe. Various clinical, clinical-pathological. epidemiological, and parasitological aspects not previously reported in this disease will be presented.

HISTORICAL.

MODES OF DIRECT MALARIA TRANSMISSION.

That malaria can be transmitted directly from man to man without the agency of a mosquito is a well-established fact (GERHARDT, 1884). The therapeutic application of this principle is best known in the artificial induction

of benign tertian malaria for the treatment of neurosyphilis (WAGNER-JAUREGG, 1917). Infected blood is simply injected directly into the recipient to be treated, intramuscularly or intravenously.

Malaria may likewise be transmitted directly from man to man, though accidentally, following the common use of apparatus for intravenous therapy in syphilis. MAC ARTHUR (1937), in a personal communication, cited an outbreak of cerebral malaria caused in this manner in Dublin during the war. "A number of men after receiving an injection of salvarsan became acutely ill. At first the trouble was thought to be arsenical poisoning, but examination of the brains of those who had died during their illness showed the cause of death to be cerebral malaria, and parasites were demonstrated in the blood of those who had survived. None of the men afflicted had been abroad. An enquiry showed that although each man had had a fresh needle for his injection, the same rubber tubing had been used for each. A soldier who received his injection immediately preceding the first man to be taken ill had been overseas, and was found to be an ambulatory case of infection with *P. falciparum*. Apparently in the course of the injection some of his blood must have regurgitated into the rubber tubing, and so the succeeding men, in addition to the salvarsan, received also an injection of infected blood."[*]

Such an outbreak, though distressing, especially if associated with fatalities, is of little importance epidemiologically. The mechanism occurs rarely, is easily amenable to remedy, and the resultant cases are controlled by adequate therapy.

Blood transfusion is occasionally followed by accidental malarial infection if the donor's blood contains plasmodia (KORABELNIKOFF, 1927; DECOURT, 1931; JANKELSON, 1931: STEIN, 1932; GUBB, 1919). The mechanism is a simple direct transmission of blood containing parasites from donor to recipient. Isolated instances of this variety of accidental transmission are of no practical or epidemiological significance, for even if the resultant infection is not recognized clinically the donor is not likely to be used frequently enough to affect a large number of recipients. The probability is that the situation would be recognized early and the donor treated, or his blood avoided for transfusion purposes.

Finally there is the method of the direct transmission of malaria among drug addicts who practise the common use of hypodermic syringes. The mechanism is simply the direct transportation of infected blood from one malarious drug addict to one or more non-infected addicts through the agency of a hypodermic syringe which is used in common by many heroin addicts.

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LITERATURE.

MALARIA IN DRUG ADDICTS.

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BIGGAM (1929) introduced the subject in 1929. In a report from Cairo, an epidemic of malaria occurring among drug addicts who practise the intra-

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[*] The same incident has been described in WENYON's *Protozoology*, p. 955.

venous use of heroin is described. This is the first record of the communal use of heroin intravenously among drug addicts. As reported by BIGGAM the use use of the drug intravenously probably is related to the knowledge that drugs given in this manner in the treatment of parasitic diseases are more effective than when taken by other routes. The mechanism for the transmission of the disease from one individual to another is simply the transportation of infected blood from a malarious addict to one or more uninfected addicts as a result of their using a single syringe during the course of more than one intravenous injection.

An interesting and outstanding feature of BIGGAM's series is the fact that in almost every case the predominant syndrome was diarrhoea and fever, and that in every case the parasite was *Plasmodium falciparum*. It is pointed out that a serious infection of the healthy population may occur if mosquitoes suitable for transmitting malaria are known to exist in the particular area under consideration.

Following this report, and a subsequent paper by BIGGAM and ARAFA (1930), the problem of malaria in drug addicts made its first appearance in America within a short time. In several very brief accounts (GEIGER, 1932; NICKUM, 1933; FAGET, 1933; FLAXMAN, 1933; EATON and FEINBERG, 1933; BRADLEY, 1934; HIMMELSBACH, 1933: APPLEBAUM and GELFAND, 1934) sporadic outbreaks are described from various parts of the United States. It is of especial note that many of these isolated groups of cases occurred in seaport or coastal cities. There is no strikingly uniform clinical picture among these cases, nor is the parasite predominantly one species of *Plasmodium*.

Then, in 1934, there appeared the report by HELPERN to which we have already referred. The subject to that time is very well reviewed and the outstanding clinical, pathological and epidemiological features from a study of 49 cases, of which 21 were fatal, are presented. The mechanism of transmission in this series is exactly that originally described from Egypt by BIGGAM. From careful contact investigations the mode of accidental transmission as a result of the common use of hypodermic syringes by malarious and non-infected addicts is definitely proven. Although a few quartan cases and one benign tertian malaria are included in the statistical analysis, the report itself is limited to those cases caused by the malignant tertian parasite (*P. falciparum*). The 21 fatal cases were all of this variety and the outstanding clinical and pathological features were those associated with the cerebral form of malignant tertian (aestivo-autumnal) malaria. The public health implications are pointed out.

AUTHOR'S SERIES OF CASES

Since 1933 there has been a slow but continued stream of admissions to Bellevue Hospital of drug addicts suffering from malignant malaria. In spite of the facts that in recent years the disease has come to be recognized and treated

early, and that its existence must be well known to the drug addict population of New York City, more than 100 individuals have died from it since 1934. This is some indication of the probable magnitude of the number of infected individuals at large. During this five-year period we have had the opportunity of observing the clinical course of this form of malaria in a fairly large group of individuals, and of carrying out parasitological and pathological investigations in this disease. It is our object to present the results of these observations and studies.

The cases studied were from the Third (New York University) Medical Division of Bellevue Hospital and from the Medical Service (New York University) of the Psychiatric Division of Bellevue Hospital. The laboratory work was carried out in the Department of Clinical Pathology of the New York University College of Medicine. Permission to present the pathological material has kindly been given by the Director of Laboratories of the City of New York and by the office of the Chief Medical Examiner of New York City.

EPIDEMIOLOGY.

Incidence.

Naturally acquired malaria is a fairly uncommon disease in the metropolitan area of New York City and that contracted in the New York area and environs is very rare. In either case the incidence as seen in hospital experience is very low.

In the five-year period 1928-1933 there were only 70 cases of malaria recorded in the whole of Bellevue Hospital. The average yearly admissions were 59,000 for that period (Table I, p. 169). In the five years 1933-1938, on the other hand, the period which is under consideration in this report, there occurred 180 cases of malaria in approximately the same number of admissions. This great difference is seen to be due to the continued admission to the hospital of drug addicts suffering from malignant malaria in the latter period.

This is borne out in the Tables (Ia, Ib, Ic, Id) by the fact that prior to 1933, when this form of malaria was first recognized, the number of cases of malaria caused by *P. falciparum* accounted for only a small number of the total, whereas following 1933 the number of cases of malignant malaria (*P. falciparum*) made up by far the majority of the total cases of malaria.

The greater number of drug addicts admitted and the greater number of deaths ascribed to malaria further indicate the increase in the 1933-1938 period as due to the now endemic occurrence of malignant (aestivo-autumnal) malaria among the drug addict population of New York City.

In 1938, for example, there were 54 cases of malaria seen in the entire hospital, of which 45 were of the malignant form (*P. falciparum*) occurring in drug addicts. There were nine fatal cases in all, but these belonged to the addict group. There were only eight cases in non-drug addicts and naturally

acquired. Of these seven were due to *P. vivax* and only one to *P. falciparum*.

It is of interest, too, to note that prior to 1933 one very rarely saw a case of malaria in the Psychiatric Division of Bellevue Hospital. The author, during a period of intimate contact with the medical admissions from 1932-1933, failed to see a single case of malaria in the whole Psychiatric Division. After 1933, however, when malaria among drug addicts appeared in New York, many subjects with the disease, especially the cerebral type, gained admission to the Psychiatric Division because of mental or nervous system symptoms, or under arrest for observation. During the period of this study more than 50 cases of malaria, all *P. falciparum* and a good number of them fatal, were observed in the medical wards of the Psychiatric Division of Bellevue Hospital.

The incidence and mortality of malaria as it exists at present in the
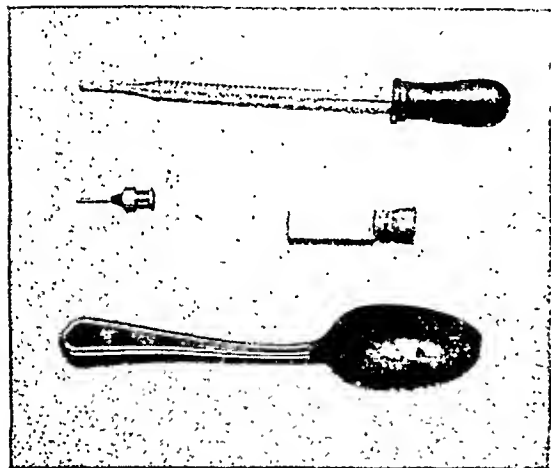
FIG. 1.

Improvised hypodermic apparatus for self administration of heroin. The drug shown in the vial is dissolved in the spoon and drawn up into the medicine dropper, which acts as the syringe.

metropolitan area of New York is therefore almost entirely due to the occurrence of the malignant form of the disease among the drug addict population who practise common use of hypodermic syringes.

Transmissions.

The mechanism for the transmission of the disease has already been suggested. As stated originally by BIGGAM, the intravenous use of heroin is related probably to the knowledge that when other drugs are taken by this method they are often more effective than when taken by other routes. Also, a greater and more quickly induced effect may be brought about in this way. The danger among known addicts of arrest for the possession of hypodermic equipment often drives them to hide their syringes or use them in common to reduce their number. Moreover, as brought out by HELPERN, the communal

use of the drug at the same time by any group of addicts makes it possible for them to obtain one or more satisfactory injections which might not be possible if they had not pooled their resources to obtain a purchasable quantity of the drug.

The technique of injection and transmission is very simple. In some cases a complete medical hypodermic syringe and needle were used. In other instances the apparatus (Fig. 1) is improvised by fitting a needle to the end of an eye or medicine dropper with the aid of a piece of rag, or newspaper or cigarette paper. The drug is dissolved, often in a teaspoon, aided by the heat from a burning match. If the injection is to be for one individual he draws the drug into the syringe and then attempts to insert the needle into one of his veins. A successful trial is known by the appearance of blood in the syringe. The drug mixed with the blood is now forced into the vein and to insure that it has all been obtained the syringe is again flooded with blood and the contents emptied into the vein. Frequently another addict is on the scene awaiting his turn to use the apparatus and to have his share of the drug. Without preliminary washing or other attempts at cleaning the syringe he proceeds to repeat the process already described. It is very obvious that if the first individual has plasmodia in his blood in fair numbers the addict following him in the use of the syringe will not only receive his share of the drug but plasmodia as well.

Undoubtedly the disease is transmitted in this way. Since attempts to demonstrate parasites from a dry, old syringe have been unsuccessful we prefer not to include the possibility that use of a syringe by addicts at relatively great intervals is an important factor in transmission. There is enough evidence that successive use of "fresh" syringes is practised commonly enough so that this method can well account for most cases of accidental infection.

The origin of the malaria seen in the addicts in this country is difficult to establish. It is impossible to state whether the various American cases originated in Egypt. Certainly many of the patients were sailors and had been overseas and in the tropics. All we can safely conclude from the data we have obtained from our own cases and from study of the other reports from the point of view of contact is that some of these individuals acted as the original foci for the various epidemics described, but at present no tropical factor is essential. The only requirement now is for some addicts to harbour a sufficient number of parasites to ensure that when their blood contaminates a syringe it will infect the individuals using it shortly afterwards.

It is interesting that many of the original cases occurred in seaport or coastal cities and that a goodly number of addicts had been sailors. In fact, one of the earliest fatal cases seen in New York was a sailor who had an Arabic inscription tattooed on his forearm! The actual contact of cases in New York with other known cases in the same area was definitely proved by HELPERN. In our own series many of the subjects questioned admitted knowing

other individuals who were or had been sick with malaria, and of having shared syringes with them.

PARASITOLOGY.

The outstanding species of *Plasmodium* in this disease is *P.' falciparum.* Although a few cases have been reported in which the parasite was either of the quartan or benign tertian variety, the majority of cases by far in the larger epidemics studied have been due to *P. falciparum.* In fact the author, in a careful study of the morphology of the plasmodium in more than 200 consecutive cases of malaria occurring in drug addicts found only one example of infection with *P. vivax* and one case in which *P. malariae* was the parasite. All the other cases were caused by *P. falciparum.* Possibly this species lends itself best to continued asexual transmission under the circumstances described for its transmission.

Morphology of the Parasite.

In the peripheral blood (Fig. 2) of the patient suffering from a moderately severe infection the characteristics of the parasite stained by the use of Wright's, or Leishman or Giemsa stain were as follows. Multiple infection of the red cell occurred as a fairly constant phenomenon. The rings on the whole were of the small, delicate variety. Marginally implanted parasites, accole, and bacillary forms were quite common. There was no enlargement of the red cell and Schüffner's granules were never seen. Occasionally, in a deeply stained smear, Maurer's dots were found. Phagocytozed pigment was often found in monocytes and in neutrophilic granulocytes. Rarely, in the peripheral blood of a moribund individual, compact deeply basophilic trophozoites and characteristic schizonts were found. Infections of considerable duration yielded typically crescentic gametocytes.

In smears prepared from the brains (Fig. 3) and other viscera obtained at autopsy the cycle of schizogony could be followed. In the capillaries of the brain numerous developing and dividing parasites were seen. These together with free merozoites and aggregates of pigment often very effectively occluded the lumen of the capillary.

In the bone marrow (Fig. 4) the striking feature associated with the growth of the parasite was the diffuse distribution of free and phagocytozed pigment. Infected red cells were numerous; young, growing, and dividing parasites were occasionally found.

In the spleen smears the features of the growth of the parasite resembled those seen in the bone marrow. Most of the red cells in the parenchyma of the organ appeared infected; much free and ingested pigment was seen, and numerous growing trophozoites and free merozoites were found (Fig. 5).

On the whole the morphological characteristics of the parasite, its cycle of

FIG. 2.—PERIPHERAL BLOOD.
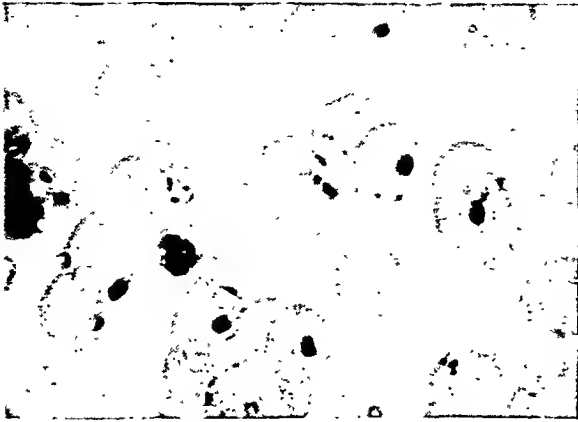
Smear of peripheral blood showing numerous red cells infected by small rings of *P. falciparum.*

Smear of peripheral blood showing multiple infection of a red cell by rings of *P. falciparum.*

Smear of peripheral blood showing phagocytic cell with ingested malarial pigment. Note a marginal implanted (accole-form) ring of *P. falciparum.*

Smear of peripheral blood showing two gametocytes of *P. falciparum.*
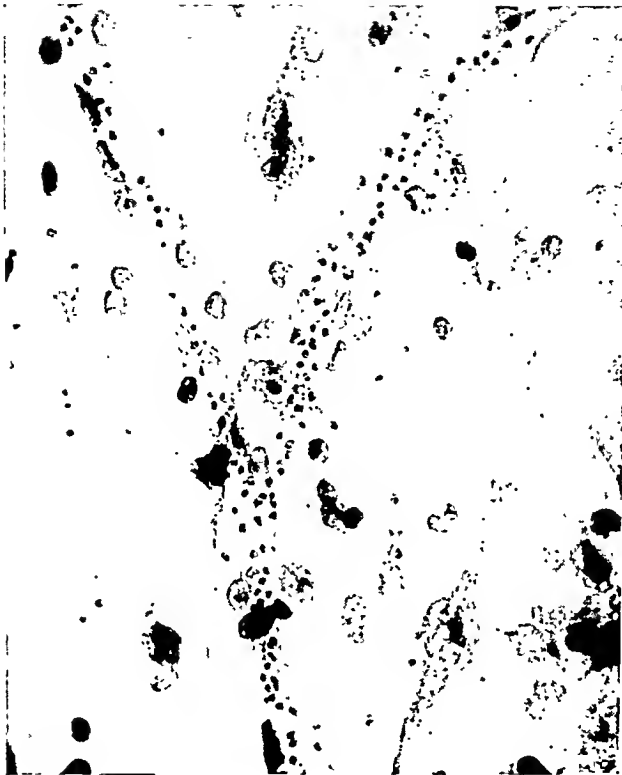
FIG. 3.—BRAIN SMEAR.

Smear of brain showing capillary almost completely filled by parasites.
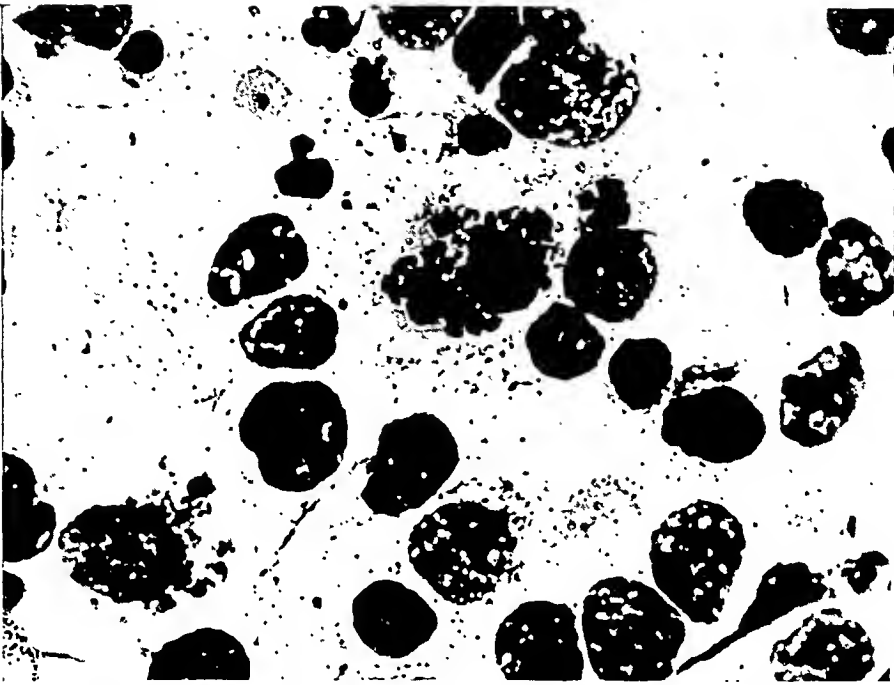
FIG. 4.—BONE MARROW SMEAR.
Smear of bone marrow showing free and phagocytosed malarial pigment.
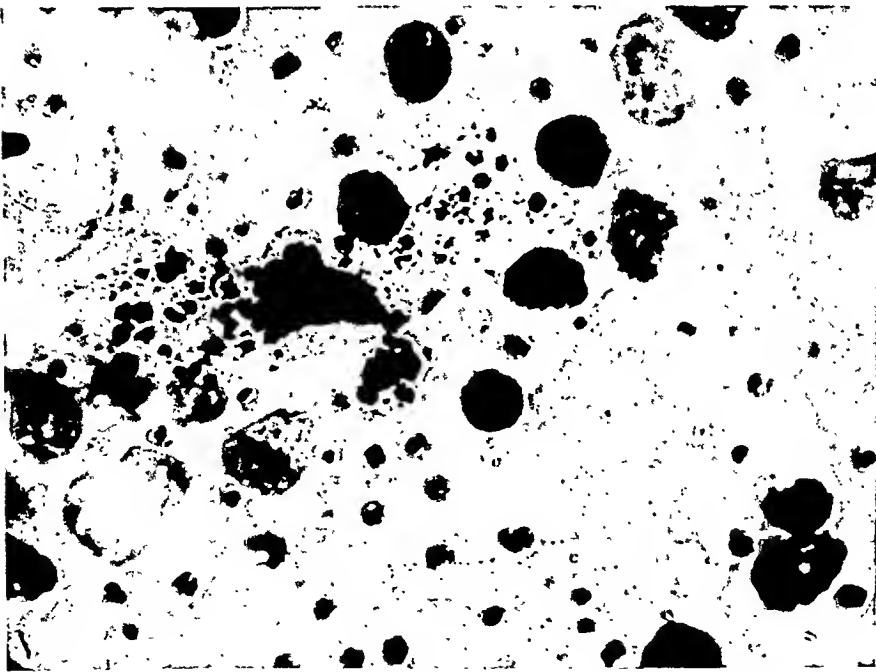
FIG. 5.—SPLEEN SMEAR.
Smear of spleen showing free and phagocytosed pigment and numerous parasites throughout.

FIG. 6.—EVIDENCE OF DRUG ADDICTION.

Forearm of intravenous heroin addict. Note the prominent vein over the forearm and in the antecubital fossa. These veins are pigmented and often scarred and partially thrombosed.

Forearm and arm of a subcutaneous heroin addict. There are numerous superficial and deep scars. The skin is pigmented and thin over healed injection sites.

development, and the changes produced in the blood and viscera were definitely typical of *P. falciparum*.

Culture.

Numerous attempts to culture the parasites obtained fresh from the blood of untreated individuals were unsuccessful. Often blood was used which contained an overwhelming infection, and occasionally moderate or light infections were tried. The method was that described by THOMSON and consists of incubating freshly infected defibrinated blood at 37° C. in tubes containing a small amount of 50 per cent. glucose solution. Citrated, oxalated, heparinized and clotted blood were likewise employed, but without success. This experience coincides with that of L. T. COGGESHALL at the Rockefeller Institute, who likewise made many attempts to culture the parasites obtained from the cases in the New York drug addict series.

Animal Inoculation.

An effort was made to transmit this species of plasmodium to a monkey (*Macacus rhesus*) by injecting 20 c.c. of fresh heavily infected blood intramuscularly into the animal. The monkey failed to show evidence of infection clinically or otherwise, and when the animal was later killed there were no signs of malarial infection in the organs. COGGESHALL, in a personal communication, informed the author that numerous attempts to infect monkeys with the parasites obtained from drug addicts were likewise unsuccessful. In the face of this and other experience elsewhere, further attempts to infect monkeys were not pursued.

Mosquito Infection.

Several observations and public health considerations made it desirable to know whether mosquitoes could be infected by feeding them on addicts who harboured the parasites. These considerations were:—

1. *The itinerant nature of the addicts.*—It is well known that drug addicts wander from city to city in search of easy contacts or "rackets" or work to insure a steady supply of the drug. It has already been mentioned that some are sailors. If they can infect suitable mosquitoes where these are abundant they are a serious menace to such areas.

2. *So-called carriers.*—Some addicts who have recovered from the disease following treatment and some at large who never had symptoms of the disease are known to harbour gametocytes in their blood. These gametocytes were observed by the author to be able to undergo direct exflagellation. If, then, mosquitoes can be infected from addicts harbouring gametocytes, they too

are a public health menace, even though they cannot transmit the disease any longer directly to other addicts. It becomes incumbent, then, on those who treat these addicts to be certain they are free of gametocytes before they are discharged, and to use suitable drugs to insure this.   It is also desirable that those who come in contact with drug addicts for any cause whatever should search their blood for gametocytes and if they are found treat them accordingly.

3. *Failure of gametogony in some cases.*—In some addicts, after recovery from the disease, it was noticed that no gametocytes appeared in the blood although no special gametocidal drug had been employed. It became interesting to ascertain whether the power of gametogony was in some way altered or whether as a result of repeated asexual transmissions for many years the gametes had lost their power of returning to their natural biological host, the anopheline mosquito. If this were so, then the drug addicts were not a menace to the general healthy population of any area, particularly a part of the country where anophelines are abundant, but were only a menace to each other *via* direct asexual transmission.

Through the courtesy of L. T. Coggeshall of the Rockefeller Institute several female *Anopheles quadrimaculatus* mosquitoes were obtained. They were allowed to feed on the thigh of a drug addict who had recovered from an attack of severe malaria which he had contracted by the method already described. The patient was afebrile and showed a moderate number of gametocytes in his peripheral blood. After the lapse of a week Coggeshall dissected a few mosquitoes each day to see whether they had become infected. The last three insects, of eight dissected, were positive, containing 15, 21, and 23 oöcysts respectively.

This is conclusive proof of the infectivity of drug addict malarious blood containing gametocytes for anopheline mosquitoes. All the speculations concerning the public health problem in relation to cases and carriers are seen to have a firm foundation in actual fact. If a sufficient number of addicts, whether recovered but incompletely treated or asymptomatic carriers of gametocytes, should accumulate in an area of the country where suitable mosquitoes are abundant a serious epidemic of malaria involving the healthy population may occur. Likewise, an epidemic may occur if a sufficient number of suitable mosquitoes should accumulate in a non-malarious area where infected addicts are abundant!

CLINICAL ASPECTS OF THE DISEASE.

The outstanding clinical features of the cases seen early in the history of this disease in New York were predominantly cerebral in nature. In fact, many of the original cases were instances of severe cerebral malaria associated with a high mortality rate.

This feature of the disease in the drug addict population in New York differed strikingly from that reported in the first Egyptian series, in which dysenteric symptoms seemed to predominate almost exclusively. Some investigators of the disease felt that this difference, and the tendency for the disease to be manifest clinically through one system complex (central nervous), suggested a specific neurotropic strain of *P. falciparum* as the aetiological parasite.

The lapse of time, however, and the distribution of the disease among a fairly large number of individuals has given us the opportunity to see the different clinical varieties of malignant malaria, including the simple, the cerebral, the dysenteric, and even the relatively uncommon "blackwater" type of *P. falciparum* infection.

The author is of the opinion that, except for the non-biological mode of transmission, the disease as it is seen in the drug addicts in New York or elsewhere simply represents the usual course of infections with the malignant tertian parasite, in no way different from that seen even in tropical Africa.

CLINICAL SYNDROMES ENCOUNTERED.

FATAL CEREBRAL CASE: COMA.

A.G., a 34 year old coloured Porto Rican male, was admitted directly to the prison ward of Bellevue Hospital from the City Prison for observation on November 23rd, 1933. No direct history was available because the patient was in coma. The transfer record indicated that the patient had been arrested for "traffic in narcotics."

On admission the patient was seen to be poorly nourished, dehydrated, and in deep coma. He was perspiring freely, with a temperature of 102° F., pulse 120 per minute, and respirations irregular and 54 per minute. The patient could not be roused by painful or other stimuli.

Examination revealed the following outstanding features : The neck was rigid. The sclerae were somewhat icteric. The pupils were small and reacted little to light. In the fundi several flame-shaped haemorrhages were seen. The pharynx was slightly injected. The lungs were clear and the heart negative except for its rapid rate. No viscera could be palpated in the abdomen. The upper extremities exhibited changing rigidities and were remarkable because the antecubital veins were very prominent (Fig. 6). This was due to numerous small pigmented puncture sites along the course of the vessels which were thickened and, on palpation, found to be thrombosed in localized areas. There was an abscess of the right forearm. The deep reflexes were hyperactive and bilateral Babinski reflexes were present.

The impression on admission was expressed that because of the history and physical evidence of drug addiction, and the knowledge that cerebral malaria occurred among addicts who shared their syringes, the probable diagnosis was malarial encephalitis.

Examination of a stained blood smear made immediately revealed a moderate number of gametocytes of *P. falciparum*, and only a few rings after prolonged search. Intensive therapy in the form of quinine by vein, by stomach tube and by rectum was begun at once on the presumption that the major diagnosis was cerebral malaria. However, diagnostic steps enumerated in the laboratory data were carried out to exclude other causes of coma. Supportive treatment was given. Fluids were administered by infusion and hypodermoclysis, yet the patient failed to rally.

Urine : amber ; specific gravity, 1·022 ; albumin, negative ; reducing substance, negative ; acetone, negative ; bile, positive 1 : 320 ; microscopic, occasional w.b.c.

Blood : R.b.c. 4,100,000 per c.mm. ; haemoglobin, 13 grammes per 100 c.c. W.b.c. 11,500 per c.mm. Differential : meta II, 18 per cent. ; polymorphonuclear neutrophiles, 70 per cent. ; eosinophiles, 2 per cent. ; lymphocytes, 7 per cent. ; monocytes, 3 per cent. Non-protein nitrogen, 32 milligrammes per cent. ; sugar, 118 milligrammes per cent. ; icterus index, 26 ; van den Bergh, direct immediate ; Wassermann, 4 +. Culture, sterile after 72 hours.

Spinal fluid : Pressure, normal ; reducing substance, present ; globulin, not increased ; 8 cells per c.mm. ; smear, negative ; culture, sterile ; Wassermann, negative ; Colloidal gold, 0011100000.

Pus aspirated from abscess of forearm : Smear, Gram-negative bacilli ; culture, *B. coli.*

Course.—The temperature remained elevated, fluctuating between 101° F. and 104° F. On the second day the patient became conscious but he was mute. His eyes followed the examiner but he could not answer questions or establish contact in any way. Repeated examinations of the peripheral blood revealed only an occasional ring form and a moderate number of gametocytes of *P. falciparum.* Splenic puncture was therefore performed. The stained smear showed an overwhelming infection. All phases of growth and schizogony of the parasite were seen, and practically every red cell seen was infected with at least one parasite. Free and ingested pigment was abundant.

The diagnosis of cerebral malaria was now certain as the major one, and the possibility that malarial infection was merely coincidental to another disease quite remote. Obviously the growth and division of the parasite was occurring in the capillaries of the viscera and brain, and so accounted for the clinical features observed as well as the paucity of parasites in the peripheral circulation.

Despite intensive specific and supportive treatment the course progressed downhill and the patient died in coma on the third day following admission.

Necropsy examination by Dr. M. HELPERN confirmed the diagnosis of cerebral malaria.

The brain appeared slate blue in colour. There were pin-point haemorrhages throughout the white matter seen on section. The contrast between the white and grey matter was striking, and the latter appeared slate coloured. Smears made from the brain, spleen, bone marrow and liver revealed the changes already described under the parasitological characteristics of *P. falciparum.* The gross and microscopic findings in the organs were those seen in acute malignant tertian malaria, and will be presented in the discussion of the pathology of this disease.

Comment.

The outstanding feature in this case was the profound cerebral involvement manifest clinically by coma. Stupor and coma often represented the only clinical evidence of nervous system involvement in a large number of the fatal cases. In many instances when the disease had progressed unrecognized to that state treatment, no matter how intensive, proved of little avail.

Appreciation of the pathogenesis of the process readily accounts for the clinical picture seen. Practically all the capillaries of the brain are occluded by agglutinated parasitized red cells, by free and growing parasites, and by a large amount of pigment. Often there is diffuse haemorrhage and oedema in the substance of the organ.

Stupor and coma were seen on admission as the only expression of cerebral involvement among the drug addicts, in ten of the fatal cases studied. Often

the individual was found lying in some hallway, or in the street, or at home. Questioning in most of these cases was futile, as the addicts could not be roused in any way. Occasionally after intensive treatment consciousness would return for a short time but good contact could not be established during the conscious interval.

Convulsions occurred rather infrequently. In one fatal case convulsions were ascribed to alcoholic poisoning, although later in the course of the patient's coma the proper diagnosis was established. In two other fatal cases convulsions were observed on admission or during the course of treatment in the hospital.

Neurological signs other than stupor, coma, or convulsions, though abundant in some of the fatal cases seen, were neither constant, localizing, or of particular diagnostic aid. Stiffness of the neck, hyperreflexia and pathological pyramidal tract sign reflexes were the most common of these findings. Occasionally changing rigidities, sucking and grasping reflexes were seen. Rectal and vesical incontinence were quite common. On the whole, the signs were suggestive of an acute diffuse meningitic or encephalitic process.

Mental symptoms or signs observed in the cases requiring admission or transfer to the Psychiatric Division were likewise not constant or diagnostic. The outstanding finding was confusion. Occasionally the patients were restive, restless, and negativistic. In most instances the confusion and apathy were considered as part of an acute inflammatory central nervous system process. If, however, when the diagnosis of malaria was established the patient was dull, or apathetic, or slightly confused, or resistive and restless, or excessively irritable, or emotionally unstable (crying), the probability of cerebral involvement was considered. Intensive therapy for cerebral malaria was instituted even though no neurological signs were found. If this was done early the individual did not lapse into the coma from which so many addicts never awoke.

Mental symptoms in malignant malaria are therefore important in suggesting cerebral involvement and early intensive intravenous therapy.

The non-fatal cerebral cases differed very little from those who died except possibly in the severity of the presenting signs, the duration of the stupor or coma before treatment was begun, and in the intensity of the treatment. In most cases if the coma was deep and of considerable standing the outcome was fatal no matter what the treatment was. On the other hand in some individuals, despite deep coma and considerable evidence of diffuse cerebral involvement, recovery was dramatic and complete shortly after intensive intravenous quinine therapy was given.

One is therefore left with no choice but to treat every patient with malignant malaria with the slightest suggestion of cerebral involvement at the earliest possible moment and in a most intensive manner. It is gratifying to the author that in the past year or more, when this practice was followed, no case terminated fatally.

GASTRO-INTESTINAL SYNDROMES.

Case 1. *Diarrhoea and Vomiting ; fatal case.*

G.H., a 24 year old negress, was a patient on the Psychiatric Division of Bellevue Hospital. Because of vomiting and diarrhoea she was transferred to a ward of the Medical Service of the Division.

When questioned she revealed that for a week she had complained of abdominal cramps, diarrhoea, and vomiting. She admitted heroin addiction by the intravenous route, and marihuana smoking.

During the examination the patient vomited frequently and also passed loose, yellowish mucoid stools. There was no fever or respiratory distress. The pulse was 96 per minute and the blood pressure 90/55 (mm. Hg.). The patient rolled around in bed complaining of abdominal pain. The abdomen was diffusely tender but no masses were felt and no rigidity encountered. Except for this and the prominent, scarred, and pigmented antecubital veins the physical examination was not remarkable.

The patient continued to vomit and have diarrhoea. Complete laboratory study, including an examination of the stool, urine, spinal fluid and the blood, showed no abnormalities and shed no light on the diagnosis. A single smear of the blood revealed no plasmodia.

The temperature, which was normal on admission, rose to 104° F. A surgeon thought there might be a pathological condition in the gall bladder. However, in spite of all symptomatic and supportive treatment the patient's condition grew steadily worse. She developed a stiff neck, exhibited sucking and grasping reflexes, and Kernig reflexes. The patient died on the 2nd day following her admission to the hospital, her condition being undiagnosed medically or psychiatrically.

Autopsy performed by Dr. MILTON HELPERN of the Chief Medical Examiner's Office revealed the cause of death to be malignant malaria !

Case 2. *Bilious Vomiting ; Surgical Intervention ; fatal case.*

H.H., a 39 year old white male, was a prisoner in the Tombs City Prison because of drug addiction. On 23rd December, 1937, he was transferred to the prison ward in the Psychiatric Division of Bellevue Hospital because of abdominal pain, vomiting and diarrhoea of 5 days' duration. The only other points in the history were drug addiction and an appendicectomy many years ago. The onset of the abdominal pain was fairly sudden and was followed by vomiting and diarrhoea. Vomiting followed each attempt to eat, and diarrhoea to the extent of twenty bowel movements daily commenced on the 1st day of the illness.

Examination showed the patient to be acutely ill. The temperature was 101° F. and the pulse 116 per minute. The extremities showed evidence of long standing intravenous drug addiction. The abdomen was tender in the epigastrium and over the liver. There were no remarkable findings reported in the laboratory data.

Course.—The patient continued to vomit and the impression expressed by the visiting surgeon was that an acute abdominal condition (intestinal obstruction, perforated viscus, etc.) was a likely diagnosis, and laparotomy was indicated. This was accordingly performed.

At operation the spleen was found enlarged to three times its usual size and the liver likewise enlarged and slate grey in colour. The mesenteric nodes were numerous and enlarged. There were omental adhesions between the peritoneum and ascending colon but no obstructions or perforations were found. These findings suggested a diagnosis of Hodgkin's disease to the operator.

The post-operative course was stormy. The patient evacuated and did poorly in spite of transfusions and other supportive measures. The temperature fluctuated in a septic manner between normal and 105° F. The serum bilirubin was elevated (5 and 2·5 milligrammes per cent. on two ocasions), otherwise the laboratory data were not remarkable. The patient died on the 28th day of his hospital stay.

Necropsy perforated by Dr. HELPERN revealed the cause of death to be malignant malaria. There was evidence of acute malaria in all the viscera, including the brain. There were no perforations in the viscera and the intestines showed diffuse deep malarial pigmentation.

Case 3. *Vomiting and Jaundice ; fatal case.*

A.B., a 52 year old white male who was a prisoner in the City Prison because of drug addiction, was transferred to Bellevue Hospital on account of jaundice and vomiting of 10 days' duration.

The patient was co-operative, though weak, and stated that a month before the onset of his illness he had had " chills and fever " and began to vomit after meals ; lately this happened more often, so that he could no longer retain even water. He added that during the past 10 days he had been jaundiced and passed light stools.

The patient appeared very jaundiced and was so weak that he could hardly speak. The liver and spleen were palpable. The temperature on admission was 99·2° F., but rose the next day to 103° F. The non-protein nitrogen was 96 milligrammes per cent. and the serum bilirubin 15·3 milligrammes per cent. Other laboratory data were not reported. The impression was that the primary cause of the vomiting and jaundice was obstructive in nature, possibly carcinoma of the head of the pancreas, but malaria was to be ruled out. The course of the patient was short and downhill. He died 17 hours after admission.

Necropsy performed by the Medical Examiner revealed the cause of death to be malignant malaria. Smears of the blood, spleen, and bone marrow contained numerous developing schizonts and gametocytes of *P. falciparum*. The viscera were deeply pigmented.

Comment.

These cases are especially significant in a clinical consideration of the disease because of the predominant rôle the gastro-intestinal signs and symptoms played during life. In other series reported in this country, particularly that seen in New York in 1933 by HELPERN, the outstanding clinical feature was associated with cerebral involvement. In fact, this suggested to him and others that the strain of *P. falciparum* encountered here might have particularly neurotropic properties. It was stressed in support of this that in BIGGAM's original report from Egypt the outstanding clinical syndrome was gastro-intestinal in nature—dysentery—and that in the New York series this syndrome was not encountered.

The author has already suggested that the present series is now large enough, and that the disease has run a sufficiently long course to express in the protean manner for which *P. falciparum* malaria is well known, without invoking numerous specific strains in any single epidemic.

In the present series diarrhoea and vomiting were frequently encountered in the non-fatal cases. Vomiting was occasionally so distressing that specific therapy was given intravenously although the patient was not critically ill and had no cerebral signs or symptoms. In several instances diarrhoea persisted throughout the acute phase of the illness but decreased promptly when the parasites disappeared from the peripheral blood.

The diagnostic implications of the fatal cases cited with marked gastro-intestinal signs or symptoms are obvious. It seems that one can best benefit from these and similar experiences by following a practice often adopted in the tropics or areas where malaria (especially malignant malaria) is endemic. This is, to give specific antimalarial therapy in those cases which are perplexing and in which, because of technical difficulties or inexperience, plasmodia cannot be found in the peripheral blood.

The author feels that this is a proper and justifiable practice to adopt among drug addicts who are critically ill and in whom a satisfactory diagnosis other than malaria has not been established. The treatment cannot be deleterious to the patient's welfare, should not interfere with a continued search for a proper diagnosis on which it may shed light by causing a dramatic therapeutic effect.

BLACKWATER FEVER

Case 1.   U.W., a 25 year old white male.

He was found lying in coma in a subway train; but could be roused by ammonia fumes, and stated that he had been drinking. For this reason he was brought to the Psychiatric Division of Bellevue Hospital where he was assigned to the care of the Medical Service of the Division.

On admission no further history was obtainable. The patient was acutely ill. The temperature was 103° F., the pulse 98 per minute, and the respirations 34 per. minute. He could be roused, but contact could not be maintained, and he soon lapsed into coma. There was evidence of intravenous drug addiction, and except for oedema of the lower extremities the admission examination revealed nothing remarkable.

A blood smear made at once revealed numerous rings and gametocytes of *P. falciparum.* The spinal fluid was normal. Other laboratory data were as follows :

Blood : R.b.c., 2,580,000 ; Haemoglobin, 7·25 grammes per 100 c.c. ; W.b.c., 12,400 ; Differential : metamyelocytes II, 17 per cent. ; polymorphonuclear neutrophiles, 61 per cent. ; lymphocytes, 18 per cent. ; monocytes, 4 per cent. Serum bilirubin, 1·6 milligrammes per cent. ; van den Bergh, direct delayed ; Nonprotein nitrogen, 27 milligrammes per cent.

Urine : Obtained by catheter, small in amount, highly acid, deep dark wine red in colour ; contained a marked amount of albumin and, microscopically, haemoglobin and red cell casts, and a moderate number of shadow red cells ; the benzidene reaction was strongly positive. This urine, and blood taken shortly afterwards, contained marked quantities of methaemoglobin. One other specimen of urine, obtained also by catheter, was dark brown, almost black, in colour, and also contained methaemoglobin.

Course.—Specific and supportive therapy was given, including glucose infusions and whole blood transfusions. The patient failed to respond, developed signs of cerebral involvement, and died 2 days after admission to the hospital.

Necropsy performed by Dr. HELPERN showed the cause of death to be malignant malaria involving all the viscera, including the kidneys and brain.

Case 2.   C.L., a 28 year old white male.

This man was removed from the Seamen's Home in New York City and admitted to the Medical Service of the Psychiatric Division of Bellevue Hospital because he was confused and disoriented.

On admission the patient was drowsy and restless. He stated that he had become sick 4 days ago with abdominal pain, nausea, vomiting, flank pain, and chills. It was later learned that the patient was a seaman who had been in South America 3 weeks before his admission, and that he had had malaria on several occasions, for which he took quinine

intermittently but not during the past few months. The patient also stated that he had shared hypodermic syringes with addicts in New York during his present stay, and that some of them had had malaria.

On examination, except for a temperature of 103° F., apathy, restlessness, and marked costo-vertebral tenderness on both sides, very little was found.

The blood smear, examined at once, contained numerous rings of *P. falciparum.*

The urine, obtained by catheter, was bright red, but when examined microscopically revealed only an occasional red cell and numerous granular casts. There was marked albuminuria, and the benzidene reaction was strongly positive. The fresh urine contained a large amount of free oxyhaemoglobin. Several specimens obtained by catheter were similar in chemical and microscopic composition, but varied in colour from deep red to brown or almost black.

Other laboratory data on admission were as follows :—

Blood : Serum bilirubin : 4·8 milligrammes per cent. ; Nonprotein nitrogen : 43 milligrammes per cent. ; Sugar : 70 milligrammes per cent. ; Chlorides : 370 milligrammes per cent. ; Cholesterol : 138 milligrammes per cent. ; Albumin/globulin ratio : 3·7/3·4 grammes per cent. ; Wassermann : 4 + ; R.b.c. 3,780,000 ; Haemoglobin 11·1 grammes per 100 c.c., W.b.c. 9,050 ; Differential : metamyelocytes I, 4 per cent. ; metamyelocytes II, 43 per cent. ; polymorphonuclear neutrophiles, 28 per cent. ; lymphocytes, 21 per cent. ; monocytes, 6 per cent.

Course.—The patient received specific anti-malarial therapy, glucose and saline infusions, and transfusions of whole blood. The urine gradually became normal in colour and contained no abnormal constituents. The patient became brighter in a few days, and except for back pain and loin pain he had no complaints. The convalescence was rapid, and the patient was discharged 13 days after admission to the hospital.

The Donath-Landsteiner reaction for the presence of autohaemolysins was negative.

Comment.

Blackwater fever is the term applied to the clinical syndrome resulting from variable degrees of spontaneous intravascular haemolysis of red cells occuring in *P. falciparum* malaria. The mechanism for its induction is unknown. However, some factors, like continued residence in hyperendemic areas, numerous reinfections, improper, incomplete and intermittent courses of therapy, and precipitating causes, as chill, pregnancy, or acute infection, are accepted as predisposing to the attack. The outstanding clinical feature is the passage of small amounts of dark red, brown or black urine containing haemoglobin. In severe attacks marked anuria, bilirubinaemia, anaemia and azotaemia are very striking. In mild attacks, however, abdominal and loin pain may be slight, and clinical jaundice not of note.

Blackwater fever occurs most commonly in hyperendemic areas of *P. falciparum* malaria. It was well known in North America in the nineteenth century, but it has become a rarity in this part of the world in recent years. Occasionally a case is seen in an American port city, but in most instances the origin of the disease is traced to the tropics and the attack to some precipitating cause shortly after arrival in the non-malarial area.

The cases cited in this report are of extreme interest. In both cases there is little doubt about the existence of haemoglobinuria and other criteria for the diagnosis of " blackwater." The second case presented was that of a seaman. He had been to the tropics several times and had had malarial attacks at least

on four occasions. His treatment was self-administered and intermittently applied. Finally, before the onset of his blackwater episode he was exposed to malarial re-infection by *P. falciparum* as a result of sharing hypodermic syringes with other drug addicts who had had malaria. At least several suitable factors are seen to exist to explain an attack of "blackwater." Reinfection directly as a result of the common use of syringes is to be favoured as the most likely precipitating cause for the attack.

The positive Wassermann reaction in this case might at first appear discrediting for the original diagnosis. If it is recalled, however, that the Wassermann is not infrequently positive in malaria, and that in this case haemoglobinuria could not be reproduced clinically by chilling, there is little cause to doubt the diagnosis of blackwater fever. Finally, the negative Donath-Landsteiner reaction and the presence of numerous parasites, *P. falciparum*, in the blood very strongly support the diagnosis of blackwater fever.

In the fatal case there was ample evidence before and after death of severe infection with *P. falciparum*. The presence of methaemoglobin in the blood and urine, associated with gross haemoglobinuria during the course of *P. falciparum* malaria in this case, leave little else to desire for establishing the diagnosis of blackwater fever. The rapidly fatal course was probably associated with the diffuse cerebral involvement.

The opportunity of seeing two cases of this disease is an unusual one because of its present rarity in this metropolitan area. It lends further support to the belief that the malignant form of malaria we are meeting among drug addicts is merely one expression of *P. falciparum* infection.

In one other case in this series bright red urine was observed, but microscopic examination of the urine proved the condition to be haematuria. Chemically and spectroscopically no free oxyhaemoglobin or methaemoglobin was found. At autopsy it was discovered that the patient had acute diffuse glomerular nephritis.

GENERAL CLINICAL CONSIDERATIONS.

1. AGE, SEX, OCCUPATION, AND PLACE OF ORIGIN.

There were only two females in this series and both died; one of cerebral malaria and the other of acute diffuse glomerular nephritis.

The males were of young and middle adult age. Most of them had no given occupation: some were sailors. The rest worked at various unskilled trades when they did have a job.

The majority of the addicts lived in and about New York. Many were born in the United States, Porto Rico, Cuba, and the West Indies. In recent years the patients have been predominantly young coloured males from the Porto Rican and Negro sections of the city. Most of them had not been out of New York for many years.

2. Onset, history, temperature.

There were no characteristic modes of onset. In most cases careful questioning after recovery revealed that for many weeks they felt ill. In some, nausea, vomiting and diarrhoea predominated. In others there were chills and fever and headache. In the majority the symptoms were very vague and consisted of a mixture of gastro-intestinal complaints together with headache, muscle pains, and chills. In a few individuals the onset was acutely pro-
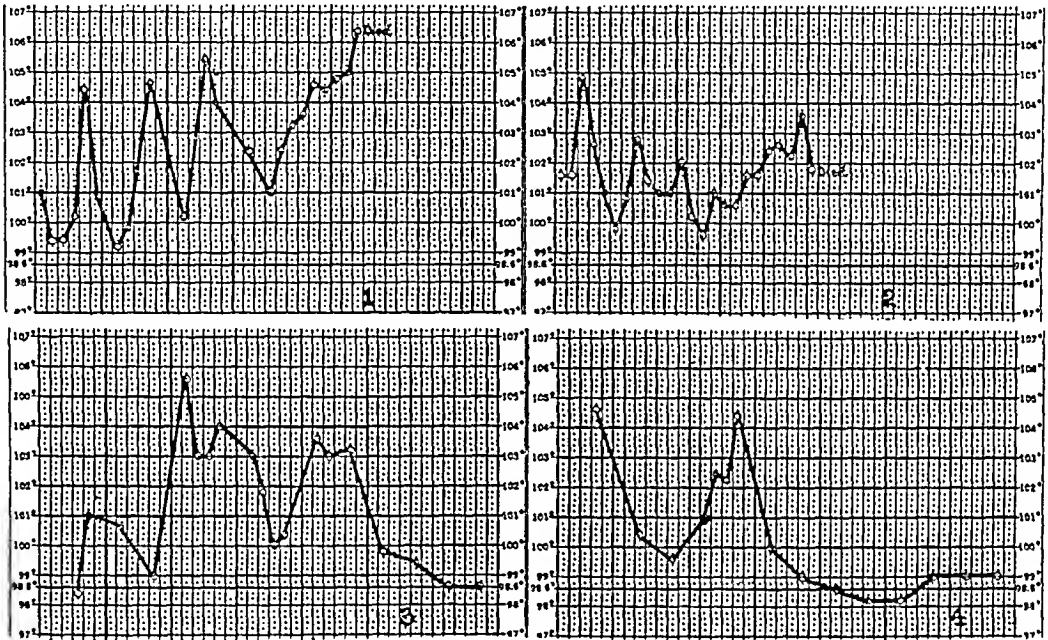
FIG. 7.

TEMPERATURE CHARTS—MALIGNANT MALARIA IN DRUG ADDICTS.

1.—Fatal case ; Quotidian Type of Fever.
2.—Fatal case ; Irregular Type of Fever.
3.—Recovery ; Double Peaks.
4.—Recovery ; Tertian Type of Cycle.

gressive, so that the patient lapsed into unconsciousness very soon. No typical onset can be given. Any "queer" complaints lasting several days in a drug addict should be regarded with suspicion.

The temperature reaction in *P. falciparum* malaria is classically unpredictable. There may be a suggestion of the tertian cycle (Fig. 7, Chart 1), but in general the temperature curve follows no given pattern. Some of the cases exhibited the double daily rise (Fig. 7, Chart 3) often described in malignant tertian malaria; others had quotidian fever (Fig. 7, Chart 1); and others were totally irregular. The fever often showed little alteration during treatment in

the fatal cases (Fig. 7, Chart 1) and in the cases which recovered there was considerable fever even several days after treatment was begun. In some cases there was little or no fever. Any fever in a drug addict should be suspected of being related to malaria no matter how little the fever may be and no matter what the chart may look like.

3. DIAGNOSIS.

Before the disease was well known the diagnosis may have been difficult. It is easy to understand why diagnoses of various cerebral syndromes were made and why a host of other medical diseases were suspected, and to condone the errors made.

The history or, in its absence, the physical evidence of drug addiction (prominent superficial veins, pigmentation, scarring, thrombosis, puncture sites, and subcutaneous and superficial ulcers or scars) is the one reliable diagnostic sign. This knowledge and a careful examination of a properly stained blood smear are usually all that are necessary to make a proper diagnosis.

Other physical signs or laboratory aids which may be helpful in the diagnosis of malaria did not occur with sufficient regularity to be entirely relied on. Splenomegaly occurred in less than half the cases; the temperature curve is not characteristic; monocytosis was found in only about one out of every six cases; and other laboratory findings are not exclusive of a great many diseases.

It has already been stated, but it may be repeated with benefit, that any acute illness or unexplained clinical syndrome in a known addict or in an individual showing evidence of heroin addiction warrants entertaining a provisional diagnosis of malignant malaria. A well-stained blood smear should be carefully and repeatedly examined for plasmodia. If as a result of inexperience or improper facilities a diagnosis of malaria cannot be made the matter of quinine therapy must be considered. Unless other specific measures are indicated, and the condition of the patient fails to improve, the author believes the drug should be given. It cannot harm the patient and should not interfere with other diagnostic measures or steps to arrive at a correct diagnosis if this should not be malaria.

4. MORTALITY.

The disease, even after early diagnosis and intensive therapy, is to be regarded as a serious one. References to the table of mortality (Table II, p. 169) will bear this out.

In the last few months of 1933, when the epidemic first was recognized in New York, most of the patients died. This can be explained by the fact that in some instances the condition was not recognized and in others, when the diagnosis was made, the disease had progressed to such a stage that treatment was of no avail.

In more recent years the mortality is somewhat reduced as a result of earlier recognition and more intensive treatment. The mortality experienced in the cases observed on the Psychiatric Division is somewhat higher. This is probably due to the fact that many of the patients seen on that service entered the hospital with the well-advanced cerebral form of the disease.

LABORATORY STUDIES.

The clinical pathology of malaria as studied in the present series is quite representative of the disease as a whole. Reference to Tables IIIA, IIIB, IIIc, IIID, and IIIE (p. 170) will bear this out.

HAEMATOLOGICAL OBSERVATIONS.

Red Cells, Haemoglobin, and Mean Corpuscular Volume.

In the haematological tables one sees that in about half the individuals studied considerable anaemia existed. In approximately 25 per cent. of the cases the red count ranged between 1 and 2·5 million cells per c.mm. The haemoglobin is likewise seen reduced in a corresponding number of individuals. and to such an extent that the colour index on the whole is near or slightly below unity. On the other hand, in many individuals, despite severe malarial infection, little or no anaemia existed.

The mean corpuscular volume was determined in twenty-four cases. The results obtained in fifteen cases suggested that the size of the red cells is usually normal or somewhat below. In nine instances, however, there was definite evidence of macrocytosis by this method (WINTROBE). Since there is no corpuscular enlargement associated with the development of *P. falciparum* another explanation must be sought for the macrocytosis. Certainly these cases are complicated by dietary insufficiency and possibly by liver damage. These factors may account for the phenomenon of macrocytic anaemia. It is of interest to note that FAIRLEY and BROMFIELD (1933) in studying the average diameter of the red cells in fourteen cases of *P. falciparum* infection (not drug addicts) by the halometer found them all within the normal range. They doubt the existence of true megalocytic anaemia in uncomplicated cases. Certainly the disease in the drug addict is not simple uncomplicated malaria.

White Blood Count and Monocytosis.

From a diagnostic point of view the white blood count and the differential formula, especially with reference to the number of monocytes, are seen to be of little value. Although leukocytosis existed in 31 cases, the total white count was within the normal range in thirty-five cases and below it in nine. Such a distribution can certainly be found in most acute febrile infectious diseases.

Monocytosis was seen only on twelve occasions of seventy-five when a differential count was done. Unfortunately this finding, so much stressed in

texts, would appear to be of little value as a diagnostic aid. The presence of ingested pigment, however, in the face of monocytosis,. is strong presumptive evidence of malarial infection.

<div align="center">BLOOD CHEMICAL OBSERVATIONS.</div>

Nonprotein Nitrogen.—The blood urea is frequently reported elevated. in malaria, especially of the malignant variety. In sixty-two cases in which the nonprotein nitrogen was determined it was between 50 and 100 milligrammes per cent. on eight occasions. In several instances kidney disease (acute or chronic glomerular nephritis) was known to exist, but on the whole the signi-ficant elevations were felt to be associated with the malarial process. The azotaemia of blackwater fever has already been commented on. Slight elevations are compatible with dehydration, vomiting. anoxaemia, anaemia, and haemo-concentration. These factors certainly existed in many of the cases in which the nonprotein nitrogen was elevated. In the cases in which recovery followed treatment and no kidney disease existed the values returned to normal very quickly.

Blood Sugar.—The blood sugar was found significantly elevated on admission only a few times. Restoration of body fluids and control of vomiting brought about a prompt fall in the blood sugar value. Severe vomiting, haemo-concentration, high fever, anaemia, and diffuse liver involvement in the course of acute malignant malaria can easily explain temporary derangement of the carbohydrate regulatory mechanism.

Serum Bilirubin.—The outstanding blood chemical finding in malaria is the elevation in the level of the serum bilirubin. If 0·75 to 1·0 milligrammes per cent. is considered the upper limit of normal, approximately 80 per cent. of the cases studied exhibited hyperbilirubinaemia.

Malaria is classified as a haemolytic disease. In the process of haemolysis colloidal bilirubin is elaborated. This substance is normally present in small amounts in the blood and is connected in the liver to crystalline bilirubin. In excessive destruction of red cells the level of colloidal bilirubin is increased in the blood and the serum bilirubin is elevated. Since this substance produces the indirect van den Bergh reaction the latter phenomenon is seen in mild or moderate haemolysis. However, the immediate reaction is also seen fairly frequently. The explanation lies, possibly, in the fact that in extreme liver envolvement and associated anoxaemia many of the polygonal cells are injured. This permits the regurgitation of changed (crystalline) bilirubin back to the blood, and accounts for the direct immediate reaction as well as the presence of bile in the urine in the cases where the renal threshold has been exceeded.

In the cases in this series, whenever the height of the serum bilirubin was considerable, the van den Bergh reaction was of the immediate type. The higher levels were associated with moderate anaemia and clinical icterus as well

as urobilinuria. The excessive destruction of blood, with its attendant increased secretion of bile pigment into the intestines, readily explains the high values obtained for the urobilinogen in the urine.

Plasma Proteins.—The total proteins were reduced below 6·0 grammes per cent. in eleven out of seventeen subjects whose plasma proteins were studied. In the main this reduction, as well as a lowering or inversion of the albumin-globulin ratio, was due to the depression in the value for serum albumin. The latter values were considerably below normal in at least fifteen of the total number studied. The serum globulin, on the other hand, was fairly normal, although definitely increased in six cases. This is of interest in relation to the positive Henry's serum flocculation test which is discussed later.

In some instances the reduction in the total protein was sufficiently great to be associated with clinical oedema. Certainly the dietary of drug addicts leaves much to be desired for completeness. One finds in the history preceding the immediate illness that the individual often partakes only of coffee and breadstuffs for weeks at a time. Nausea, vomiting, diarrhoea, poor protein intake, diffuse malarial liver involvement, and blood destruction may account at least partly for the derangement in the plasma protein values recorded.

SPINAL FLUID.

It is surprising that in the face of severe involvement of the brain, which occurred so often, relatively few changes were observed in the composition and nature of the spinal fluid.

The fluid, on the whole, was clear and in the cases in which the pressure was measured the range was within the normal limits. Pleocytosis was seen only twice, and bloody or xanthochromic fluids were noted twice each, despite the fact that diffuse petechial haemorrhage in the brain was seen often at necropsy. Globulin was seen increased qualitatively only ten times. No positive Wassermann reactions were seen, and the colloidal gold curves were normal, on the whole.

The chemical values for protein, sugar and cholides were strikingly altered, and the few changes seen may well reflect the altered values of these substances in the blood.

SEROLOGICAL DATA.

Complement Fixation.—The Wassermann reaction was strongly positive (4 +) in twelve cases, weakly positive twice, and doubtful on three occasions. Even if one conceded a greater incidence of syphilis among drug addicts one would hardly expect such a relatively high percentage of syphilis if the positive Wassermann were interpreted in that way.

That complement fixation results in malaria is well appreciated in the tropics. Unfortunately in most of the cases studied the routine Wassermann was not repeated. However, in two subjects in whom the Wassermann was 4 +

at the height of the malaria, the reaction was negative after recovery. Two weakly positive and two doubtful reactions likewise became negative after recovery from active malaria.

It is well appreciated that positive reactions in the Wassermann test may occur in conditions other than syphilis. We have observed this in haemolytic phenomena and in sera in which the bilirubin is considerably elevated. Malaria is certainly a classic example in which both these conditions exist. The positive Wassermann in malaria is well known and is re-emphasized in the findings in this series.

Serum Flocculation Test for Malaria.—The distilled water modification (CHLORINE, 1937) of Henry's serum flocculation test for malaria was carried out in ten cases. The technique consists of preparing a series of dilutions of serum in distilled water and observing the mixture for the presence of flocculation. In the original Henry test melanin apparently served to accentuate the visibility of the phenomenon.

Positive results were obtained in all cases tested in dilutions varying from 1 : 200 to 1 : 1,200. Normal controls rarely exceeded dilutions of 1 : 40 and never reached a dilution of 1 : 100. It is now believed that the mechanism is related to an increase in some of the globulin fraction in the blood serum. It is of interest that in six of our cases the serum globulin was elevated.

Although the test is relatively simple, the author performed it more as an academic matter than as a diagnostic procedure, since in all cases the diagnosis could be established by a satisfactory examination of a stained blood smear. The test may be of value if plasmodia cannot be found, or in latent or unusual forms of malaria. The author's experience with the test is too limited, however, to permit his evaluation of it as a diagnostic test.

URINARY FINDINGS.

Most of the data recorded in the table of urinary findings (Table IIIE) are not very striking.

Albumin, casts, and red cells in excess may be found in many acute infectious diseases. One need not invoke malaria to explain these findings, but one knows from pathological observations that haemorrhage occurs in the kidney, and that the parenchyma may be involved in the malarial process.

Acetone undoubtedly was associated with marked dehydration, vomiting, and diarrhoea, or starvation.

Reducing substance was found on five occasions. One cannot say whether drugs other than sugar did not account for it. We have already stated, however, in relation to the blood sugar findings, that diffuse cerebral and liver involvement, dehydration, starvation, vomiting, and so forth, may explain temporary imbalance in the carbohydrate metabolism.

The presence of bile in the urine is seen associated with the increased serum

bilirubin following excessive destruction of blood. The excess bile pigments entering the intestional tract account for the great concentration of urinobilinogen found in the urine. Finally, percolation of altered bilirubin through injured polygonal liver cells results in hyperbilirubinaemia of crystalline bilirubin. This substance appears in the urine as bile when its blood concentration exceeds the renal threshold for it.

Haemoglobinuria appeared in the two cases of blackwater fever cited. In this disease there is intravascular corpuscular lysis with the liberation of oxyhaemoglobin into the plasma. Methaemoglobin, which appears to arise from the oxyhaemoglobin in the plasma (FAIRLEY), is found in the urine when the blood level exceeds the renal threshold for it.*

PATHOLOGY.

All necropsies were performed under the auspices of the Office of the Chief Medical Examiner of New York, most of them by Dr. MILTON HELPERN of that office.

The pathological findings in the present series do not differ, in general, from those reported in HELPERN's original paper. For the details of the pathology of malaria the reader is referred to any of the standard systems of pathology (HENKE and LUBARSCH, 1926; THOMSON and ROBERTSON, 1929) and for a digest of the findings in these cases to Dr. HELPERN's report. A few brief notes will suffice for the purposes of this report.

The cause of death in the majority of the fatal cases was due to overwhelming infection and diffuse cerebral involvement. In a few cases complications directly related to malaria, such as blackwater fever, undiagnosed intestinal syndromes malarial in nature, and surgical intervention, were responsible for death. In several other instances complications not related to malaria, such as acute or chronic diffuse glomerulo-nephritis, heart disease, and pneumonia, were mainly the cause of death. In one instance a complication not related to malaria but rather to drug addiction was responsible for death. This was an addict who developed a haemolytic streptococcus bacteraemia.

In the cerebral cases of considerable duration the striking features in the brain were the gross discoloration (greyish brown), the very apparent demarcation between the cortex and the white matter on section, and the diffuse punctate type of haemorrhage throughout the white matter. In the capillaries of the brain smears parasites in all stages of growth and division, free and ingested pigment, were seen. These features have already been described.

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*More recently, FAIRLEY and BROMFIELD (These *Transactions*, 31, 139) have shown that the pigment in the plasma in cases of blackwater fever, formerly described as methaemoglobin by themselves and other workers on the subject, is not methaemoglobin but a new pigment, methaemalbumin (pseudo-methaemoglobin). Methaemoglobin is found only in the urine in blackwater fever.—*Ed.*

Microscopically the details of the gross examination were accentuated, and in addition small "ring" haemorrhages and the classical Durck granulomas were observed. Parasites and pigment were found free and ingested in the vessels which were dilated and often occluded.

The bone marrow received special study. Although grossly the marrow often appeared deep red in colour there was little correlation of the gross appearance and the changes observed in stained smears. Numerous preparations were made from many cases of varying severity and studied after combined Wright and Giemsa staining.

On the whole the bone marrow was considered primarily of the normoblastic hyperplastic type. This hypertrophy of the red marrow is apparently in response to the haemolytic destruction of the red cells which takes place as a result of the growth of the parasite and the phagocytosis of parasitized and normal red cells in the spleen, liver, brain, and bone marrow. The marked normoblastic response accounts for the spontaneous reticulocytosis often seen in malaria before treatment for the disease or anaemia is begun. This, however, is not specific response, since it may be seen in any of a number of haemolytic diseases.

Occasionally large numbers of megaloblasts were encountered, so much so that one was reminded of the marrow of macrocytic hyperchromic anaemia in relapse. This type of red marrow response, associated with improper maturation of the red cells, may account for the isolated cases of macrocytic anaemia seen in this form of malaria.

Except in the very acute cases of short duration, evidence of marked phagocytic activity was seen in the smears studied. Many macrophage cells were counted which contained large amounts of pigment or parasites. Phagocytosis of parasitized and free red cells was common. Free pigment and parasites and forms in all stages of growth and schizogony were seen.

There were no constant changes referable to the leucoblastic elements of the marrow. The author failed to notice evidence of hyperplasia of these elements or of maturation arrest. On the contrary, there often appeared to be increased white blood cell formation, but the general impression was gained that the marrow was hyperactive in all respects, although primarily in the normoblastic direction. The megakaryocytes appeared numerous and occasionally the platelets seemed increased. The livers and spleens were generally enlarged and of the slate or blue-grey malaria colour. The microscopic sections revealed the usual changes of acute malaria, viz., pigmentation, pulp hyperplasia in the spleen, and phagocytic activity of the Kupffer cells in the liver. The smears showed free and phagocytozed pigment and parasites, and all stages of growth and development of the parasites.

The outstanding feature of the general examination of the bodies was the evidence of intravenous or subcutaneous drug addiction.

TREATMENT.

The treatment which is followed at present is the result of experience gained from following the course of the disease in at least 100 cases during the past six years. In this period of time the effect of various courses of treatment and different drugs have been observed. No attempt will be made to evaluate these courses or drugs. Our purpose is to outline that treatment which we have found to be most effective in producing a rapid and satisfactory recovery from the disease with a short period of economic disability or hospitalization, and which frees the subject from being a public health menace.

PREDOMINANT CEREBRAL INVOLVEMENT.

In severe coma the object of treatment should be to produce and maintain a high concentration of quinine in the circulating blood and to restore body fluid and salts to somewhere in the range of normal. To this end we suggest that quinine-dihydrochloride be given intravenously (0·6 gramme in 10 c.c. of saline) every 4 hours for at least 24 hours during the day and night. No effort is made to give food or drugs by mouth or stomach tube. Fluids are given parenterally in the form of saline and glucose infusions and clyses (usually 2,000 to 5,000 c.c.) until urine specimens obtained every 8 hours by catheter have a specific gravity of less than 1·015 to 1·010 and are free of acetone. If anaemia or jaundice is severe, or if the urine is bright red or brown and contains oxyhaemoglobin or methaemoglobin, 500 c.c. of whole citrated blood is given as an infusion and repeated within 12 to 24 hours if evidence of marked haemolysis persists.

On the 2nd day, or as soon as clinical improvement is noted, the interval of intravenous quinine injections is lengthened so that 0·6 gramme is given every 6 hours. On the 3rd day, no matter how well the patient looks or feels, quinine-dihydrochloride is given intravenously every 8 hours, and on the 4th day every 12 hours.

In this manner it is seen that a course of quinine therapy is limited to 4 days, during which time the drug is administered exclusively by vein. As soon as the patient can take fluids by mouth he is encouraged to do so at intervals. No other drugs are given by mouth. Enemas are given daily after an initial calomel purge. The diet should be light and mostly carbohydrate. No attempt should be made to withhold heroin or morphine, in order to avoid the intestinal or nervous complications of drug withdrawal.

The efficacy of treatment is not judged during this acute phase by the temperature or the number of parasites found in the blood. The next phase of treatment consists of giving atebrin, 0·1 gramme three times daily, for 5 days. During this time the patient is placed on convalescent care and a high calorie diet. Iron in the form of ferrous sulphate, 0·3 gramme three times

daily, is also given.   If, at the end of the course of atebrin, gametocytes are
seen in the peripheral blood smears, plasmoquine (one tablet 0·2 gramme three
times daily) is given for 4 days.   The patient is instructed to return to a
follow-up clinic for a check-up examination and further treatment for anaemia
or other conditions.

In our experience this has been a very satisfactory form of treatment.   Drug
addicts do not object to intravenous medication, and in this way complications
associated with gastric or intestinal symptoms are avoided.   If it is desired to
use quinine alone it may be given on the 3rd day (quinine sulphate in capsules
0·65 gramme three times daily) and continued for the remainder of the 1st
week.

MILD CEREBRAL INVOLVEMENT.

If slight evidence of cerebral involvement is found in the form of mental
symptoms (confusion, dullness, apathy, etc.), or a few neurological signs are
found, the 1st day's regimen of the course for coma is followed.   Modifications
may be made if there is no dehydration and the patient is not critically ill.
It is our opinion, however, that if intensive intravenous therapy is given early
the severe coma state may be avoided.   On the 2nd day quinine may be
given every 8 hours, and on the 3rd day the course of atebrin is started or
quinine is given by mouth for a week.   We favour the use of atebrin.   Other-
wise the treatment is as outlined.

ACUTE INTESTINAL SYNDROMES.

When vomiting or nausea are severe, or when diarrhoea is distressing, we
suggest the use of quinine-dihydrochloride (0·6 gramme) intravenously every
8 hours for 2 to 3 days.   The stomach should be washed and a purge
given, after which daily enemas should be given. Fluids should be supplied
parenterally by infusion and clyses containing glucose and saline. Nothing is
allowed by mouth except broken ice for a day or two.   On the 3rd day
atebrin is started, and after that treatment as already outlined is continued.
A light diet is carefully begun and gradually increased.

BLACKWATER FEVER.

Much has been written about the treatment of blackwater fever.   It is not
our purpose to review the subject.   The indications for treatment in a drug
addict with malaria in whom "blackwater" is suspected are to combat shock
and to replace blood and body fluids by infusions and transfusions.   When the
acute haemolytic and azotaemic phase has passed and parasites are found in the
blood a course of atebrin is given.

SIMPLE MALARIA.

In the uncomplicated case with few symptoms or signs atebrin is started at once. The treatment is very simple and lasts only a week or two, depending on whether plasmoquine is given as a gametocide. Atebrin is given in the dosage and time already outlined, for 5 days. Dietary and iron therapy are combined to correct anaemia if it exists. If the addict wishes to withdraw from the drug habit, he is tided over withdrawal symptoms by whatever means are at hand.

LATENT CASE, AND CARRIER.

If in the routine examination of a drug addict asexual forms of plasmodia are found in his blood, he is given a 5-day course of atebrin (0·1 gramme *t.i.d.*) and plasmoquine for 4 days after gametocytes appear.

If only sexual forms (gametocytes) are found, plasmoquine alone is given for 5 days or until no more parasites are seen.

RELAPSE AND REINFECTION.

Following treatment and discharge from the hospital, several patients returned in a fairly short time with symptoms of malaria and parasites in their blood. It is difficult to accept the statements of drug addicts, but in two cases non-exposure was fairly certain after the original treatment. In one patient, who was transferred from one ward to another after recovery, relapse took place less than 2 weeks after the original treatment. This patient had received only quinine. In a second individual, who was sent to a convalescent home in the country, relapse occurred within 3 weeks after recovery. This patient had received a course each of quinine, atebrin, and plasmoquine, and was free of symptoms, parasites, and the drug habit when he left the hospital. There were no other addicts at the country home where the patient was sent, and the month was April, with low temperatures almost daily. It seems fairly certain that this patient was not exposed to infection naturally or artificially.

These cases are of particular interest especially in the face of evidence that the relapse rate for atebrin is less than that for quinine; also, because relapse occurred in spite of intensive therapy. It emphasizes the desirability of post-convalescent observation.

The matter of reinfection is somewhat different. Many drug addicts resorted to the same practices after recovery and discharge from the hospital despite a severe illness. As a consequence, some returned at a later date on another attack of acute malaria. They admitted common use of syringes with other addicts, and undoubtedly reinfection was the mechanism for the reoccurrence of symptoms and parasites. Ironically, a few addicts who recovered on the original admission died from the second episode of the disease. Unfortunately the immunity of induced malaria is extremely short-lived.

EFFECTS OF TREATMENT.

It is very difficult to evaluate the effects of treatment in this particular form of malaria among drug addicts. Lower mortality rates in recent years might be ascribed to the fact that addicts as well as hospital physicians know of the disease. As a result, patients may present themselves early for treatment, and likewise a diagnosis is made fairly soon, so that treatment is instituted shortly after admission to the hospital. Nevertheless, in 1938, in spite of treatment and the knowledge mentioned, there were six deaths in thirty-five cases admitted to the Psychiatric and Third Medical Division of Bellevue Hospital. This is still a high death rate, although it is much less than the 50 per cent. mortality experienced during the first year after the disease was recognized.

There is no intention of correlating mortality and treatment. The author merely wishes to point out that the disease is still a serious one, and that all efforts for early and intensive therapy should be applied.

SUMMARY, CONCLUSIONS, AND SUGGESTIONS.

1. New York City is an endemic area for the occurrence of malignant tertian malaria (*P. falciparum*). The disease occurs almost exclusively among heroin drug addicts who practise the common use of hypodermic syringes. Infection is direct from man to man.

2. The parasite is *P. falciparum*, and after five years of asexual transmission from man to man it is still capable of infecting anopheline mosquitoes.

3. There is local *Anopheles quadrimaculatus* production in the New York City area and environs.

4. The case load including gametocyte carriers is probable considerable.

5. These factors if properly combined constitute a serious menace to the healthy population of New York in the form of an epidemic of *P. falciparum* malaria.

6. Drug addicts recovered from malaria should be under legal jurisdiction of the City Health Department.

7. All available contacts with drug addicts should be explored, and latent or asymptomatic or " carrier " cases treated.

8. Treatment should include the use of an effective gametocidal drug.

9. Clinically and pathologically the disease manifests all the characteristics of malignant malaria as it occurs in the true tropics, including the various cerebral, intestinal, and haemoglobinuric syndromes.

10. Early diagnosis and intensive therapy are still followed by a fairly high mortality rate.

11. The outstanding diagnostic feature is the history or evidence of drug addiction. On this evidence, obscure syndromes in addicts should warrant quinine therapy.

TABLE I.
STATISTICS ON THE INCIDENCE OF MALARIA.

A.—Bellevue Hospital.	1928-33.	1933-38.
Average yearly admissions	59,000	61,940
Average yearly drug addicts	87	137
Average addicts per 1,000 admissions per year ...	1·5	2·2
Total malaria admissions	70	180
Average malaria per 1,000 admissions per year ...	1·2	2·95

D.—Types of Malaria, Bellevue Hospital, 1938.	Non-addicts.	Addicts.
P. vivax	7	1
P. falciparum	1	45
Total	8	46
Fatalities	0	9

B.—Third (New York University) Medical Division.	1928-33.	1933-39.
Drug addicts	32	120
Total malaria	20	42
Malaria in drug addicts...	0	34
Malarial fatalities ...	0	8

E.—Psychiatric Division, Bellevue Hospital, 1938.	
Number of admissions	26,210
Drug addicts	71
Drug addicts with malaria ...	27
Malaria fatalities	4

C.—Types of Malaria (Third Medical Division).	1938-33.	1933-39. Non-addicts.	1933-39. Addicts
P. vivax	12	6	1
P. malariae ...	0	0	1
P. falciparum ...	2	1	32
Undetermined ...	2	1	0
Diagnosed on history only ...	4	0	0
Total ...	20	8	34
		42	
Fatalities	0	0	8

F.—New York City, 1938.	
Type of Malaria.	Number of Cases.
P. vivax	24
P. falciparum	50
P. malariae	2
Unknown	16
Mixed	1
Total	93

TABLE II.
MORTALITY IN MALIGNANT MALARIA IN DRUG ADDICTS.

Year.	Psychiatric Division. Cases.	Deaths.	Third Medical Division. Cases.	Deaths.	Combined. Cases.	Deaths.
1933 ...	5	3	3	3	8	6
1934 ...	2	1	7	2	9	3
1935 ...	10	4	2	0	12	4
1936 ...	4	2	4	0	8	2
1937 ...	11	4	3	1	14	5
1938 ...	23	4	12	2	35	6
Total ...	55	18	31	8	86	26

TABLE III.

LABORATORY STUDIES OF MALIGNANT MALARIA IN DRUG ADDICTS.

A.—HAEMATOLOGICAL DATA.

Red Cells.

R.B.C., millions.			Cases.
1–1·9	...	...	2
2–2·9	...	...	19
3–3·9	...	...	19
4–4·9	...	...	27
Over 5	...	...	7

Haemoglobin

Grammes per 100 c.c.		Cases.
Less than 5	...	2
5–10	...	32
10·1–15	...	36
Over 15	...	3

Mean Corpuscular Volume.

Cubic microns.		Cases.
Less than 80	...	6
80–94	...	9
Above 94	...	9

White Cells.

Total count, Thousands per c.mm.		Cases.
Less than 5	...	9
5–10	...	35
Over 10	...	31
Lowest count	...	3,250
Highest count	...	46,000

Monocytosis.		Cases.
Present	...	12
Absent	...	63

B.—BLOOD CHEMICAL DATA.

Non-Protein Nitrogen.

Milligrammes per cent.			Cases.
20–35	...	...	41
36–50	...	...	13
51–100	...	...	8

Blood Sugar.

Below 100	...	23
101–150	...	23
151–200	...	6

Serum Bilirubin.

0·5–1·0	...	...	17
1·1–2·0	...	...	15
Above 2·0	...	18

Van den Bergh (Direct Reaction).

Result.			Cases.
Immediate	...	...	18
Delayed	...	...	16
Negative	...	...	13

Plasma Protein.

Serum Albumin.

Grammes per 100 c.c.		Cases.
2·0–3·0	...	...	9
3·1–3·7	...	...	6
Over 4	...	...	2

Serum Globulin.

Grammes per 100 c.c.		Cases.
1·5–2·8	...	...	11
2·9–3·5	...	...	6

A/G Ratio.

Normal	...	...	6
Reduced or Inverted	...	11

TABLE III.—*contd.*

C.—SPINAL FLUID DATA.

					C.—SPINAL FLUID DATA.		

Clear 23

Bloody... 2

Xanthochromic ... 2

Cells (more than 10 per c.mm.) 2

Globulin present ... 10

  ,,   absent ... 15

Reducing substance—

  Present 25

  Absent 0

Wassermann negative 25

  ,, positive... 0

Colloidal gold curve normal 25

Chemical.

Protein, mg. per cent.			Cases
5–15	...	...	6
16–30	...	...	1

Sugar.			Cases.
40–60	...	...	3
61–90	...	...	5

Chlorides.			Cases.
Below 700	...	...	5
700–750 ...		...	4
Above 800		...	1

D.—SEROLOGICAL DATA.

Wassermann Reaction.

Result.			Cases.
Strongly positive	...		12
Weakly positive	...		2
Doubtful	...	...	3
Negative	...	...	46

Modification of Henry's Serum Flocculation Test for Malaria.
Positive Results.

Dilution.			Cases.
1 : 200	...	...	4
1 : 450	...	...	3
1 : 1000	...	...	2
1 : 1200	...	...	1

E.—URINARY FINDINGS.

Data from 80 Admission Examinations.

Examination.			Positive.
Albumin	...	...	27
Acetone	...	...	5
Reducing substance	...		5
Bile	...	...	7
Casts ...	...	...	12
Red cella in excess	...		10
Methaemoglobin	...		2

Urinobilinogen.

Dilution.			Cases.
1 : 20 ...	...	...	7
1 : 40 ...	...	...	4
1 : 80 ...	...	...	7
1 : 160	...	...	5
1 : 320	...	...	2
1 : 1,000 and above	...		2

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was not ruptured as it was " such a very large spleen ". In 1927 there was prolonged fever (104° F.) lasting 6 weeks. In 1928 severe anaemia again developed (haemoglobin : 30 per cent.), this time with dyspnoea and cardiac complications. Partially cooked liver was administered with dramatic improvement and his medical notes showed that the haemoglobin rose to 75 per cent.

In 1931, fever recurred with epistaxis ; an enlarged spleen was again recorded. A blood transfusion was given. On clinical grounds treatment for kala-azar was commenced with neostibosan, but as the temperature came down after the third injection these injections were discontinued. He recovered in 2 months' time.

In 1932, he again had fever and anaemia ; on this occasion benign tertian parasites were demonstrated. Oral liver extract (P.D. & Co.) was given with good results.

Subsequently he went to school in Bangalore for 5 years. Though there was a history of an attack of jaundice he was little, if at all, troubled with fever during this period. Throughout his boyhood in India he appears to have had a reasonable diet and there was nothing to suggest a primary dietary insufficiency as a factor in his ill health.

II.—Grave Haemolytic Hypochromic Anaemia after Arrival in England.

He arrived in England on 7/1/38. During the next few months he worked hard, lived in a tenement with many stairs to climb and had a diet containing milk and eggs but little or no red meat, no vegetables and only a small quantity of fruit. From March onwards he noticed that he was gradually becoming sluggish and drowsy ; there was some loss of appetite, increasing dyspnoea on exertion and swelling of the feet after walking. He was admitted to the Hospital for Tropical Diseases on 25/8/38.

Physical Examination.

The patient was very pale and weak ; teeth excellent ; no pyorrhoea ; the tongue was pale and smooth. Fever with a temperature of 100° F. occurred in the evenings. Nails pallid, but otherwise normal ; no clubbing of fingers.

Thorax.—The apex beat was in the fifth interspace $4\frac{1}{2}$ inches from mid line. There was pulsation in veins of neck, gallop rhythm, a to-and-fro murmur at the apex and a systolic murmur in the aortic area transmitted to the neck. Blood pressure : S/D = 130/? ; the diastolic pressure was difficult to determine. Lungs : clear.

Abdomen.—Soft relaxed muscles. An enlarged hard spleen extending to the level of the umbilicus (2, Hackett) was present ; freely moveable ; not tender.

At this time the liver was not palpable but it became demonstrably enlarged later.

Central Nervous System.—No abnormality ; reflexes brisk.

Skin.—Naturally pigmented ; it was dry, harsh in consistency and in certain areas where pigmentation had increased, as over the elbows and knees, the appearance was somewhat reminiscent of quiescent pellagra. No oedema.

Laboratory Investigations.

Blood.—R.B.Cs = 1,200,000 per c.mm. ; haemoglobin = 15 per cent. (Haldane) ; colour index = 0·6 ; M.C.V.= 78·1 c.μ. Red cells showed hypochromia, anisocytosis,

punctate basophilia and Howell-Jolly bodies. There were nucleated red cells, six erythroblasts and three normoblasts being counted per 100 leucocytes. Leucocytes = 7,000 per c.mm. ; neutrophil granulocytes = 36 per cent. ; lymphocytes = 60 per cent ; monocytes = 1 per cent. ; eosinophiles = 2 per cent. ; basophiles = 1 per cent. Blood group III (Moss). Van den Bergh—indirect reaction, positive (4 units), direct reaction, negative. Methaemalbumin demonstrated in layer of serum 2 cm. thick. Schumm's ammonium sulphide test positive.

TABLE.

DATA RELATING TO SIZE OF ERYTHROCYTES.

Date.	R.B.C.s (Millions).	Hb. Per cent.	M.D. (μ)	σ (μ)	v (Per cent.)	Megalocytes. (Per cent.)	Microcytes. (Per cent.)	M.C.V. (c.μ)	M.C.T. (μ)	$\dfrac{\text{M.C.T.}}{\text{M.C.D.}}$
Before Treatment (13/9/38)	1·17	16	7·113	0·75	10·54	0·6	2·0	78·1	—	—
During Treatment (11/11/38)	2·93	40	7·509	1·04	13·94	12·0	1·2	100*	2·26	0·3
(29/11/38)	4·34	54	6·933	0·83	11·96	0·6	5·6	73·7	1·95	0·28
After Splenectomy (8/2/39)	5·54	70	7·20	1·03	14·30	3·4	8·6	68·6	1·68	0·23
(21/11/39)	4·20	60	8·924	1·297	14·53	59·0	0	85·7	1·37	0·15

*A reticulocytosis of 18·2 per cent. accounts for the transient megalocytosis and increased cell volume on this date.

M.D. = mean diameter. σ = standard deviation. v = variability. M.C.V. = mean corpuscular volume. M.C.T. = mean corpuscular thickness. M.C.D. = mean corpuscular diameter.

$\dfrac{\text{M.C.T.}}{\text{M.C.D.}}$ = ratio of above.

Urine.—Contained a trace of albumin, a small quantity of urobilin, but no bile salts or pigments.

Faeces.—Dark brown colour due to excess of stercobilin. The occult blood test was negative. No ova or protozoa.

The Formol-gel reaction, the Wassermann and the Kahn reactions were negative.

On 29/8/38 the mean corpuscular fragility was 0·37 per cent. NaCl, and at this stage of the illness no target cells were demonstrable in the blood films. Subsequent increases in fragility are depicted in Graph 2. Neither the fractional test meal nor the Price-Jones curve was done immediately, but later sluggish HCl secretion was demonstrated after a histamine injection (maximum 40 c.c. N/10 HCl at ¾ hour). The Price-Jones curve on 13/9/38 was as follows : M.D. = 7·113μ ; σ = 0·75μ ; v = 10·54 per cent. ; megalocytes = 0·6 per cent. ; microcytosis = 2·0 per cent. No target cells seen ; other changes as already described. The changes in subsequent Price-Jones curves are given in the table.

PROGRESS NOTES AND TREATMENT.

The writer had been very impressed in Macedonia with the frequency of haemolytic anaemia of both normocytic and megalocytic type associated with chronic malarial splenomegaly and the hypertrophy of the R.E. system generally, and the case was provisionally placed in this category.

Failure of Iron Medication and Parenteral Liver Therapy.—On 31/8/38 a blood transfusion (1 pint) was given and iron therapy commenced in the form of haematinic plastules (simplex), 2 grammes of ferrous sulphate being administered daily. Owing to the previous benefit derived from liver therapy in India, 2 c.c. of reticulogen* (Eli Lilly) were injected daily for 10 days commencing on 8/9/38. Vitamin reinforcement of the diet with multivite pellets (B.D.H.), 6 pellets daily, was also instituted. No haemopoietic response ensued (Graph I). Later, atebrin, 0·1 gramme t.d.s., p.c., was given for 3 days, but was discontinued as it appeared to upset the patient. Despite these measures and two more blood transfusions no improvement in the anaemia was recorded ; the reticulocytes failed to rise, fever increased (102° F.) and cardiac decompensation ensued. The liver became palpably enlarged, congestive signs were present at the bases of the lungs and oedema of the feet and ascites developed. X-ray examination showed marked generalized enlargement of the heart shadow with congestive changes in both lungs. Dr. EVAN BEDFORD reported gross general cardiac enlargement with a prominent pulmonary artery and conus. There was a systolic murmur and gallop rhythm at the apex with an accentuated pulmonary second sound. Blood cultures were negative. The cardiac condition was regarded as due to hypertrophy and dilatation secondary to fatty changes in the heart muscle caused by chronic anaemia.

Response to Crude Liver Extract Therapy and Iron Medication.—By 17/10/38 the condition was desperate. R.B.C.s = 865,000 per c.mm. ; haemoglobin = 15 per cent. ; reticulocytes = 0·2 per cent. Van den Bergh : indirect positive (2·5 units). Serum proteins = 5·39 per cent. ; albumin = 2.34 per cent. ; globulin = 3·05 per cent. Faecal fat : total = 18·5 per cent ; fatty acids = 12·0 per cent. ; neutral fat = 6·5 per cent. ; splitting normal. Another transfusion was given and it was now decided to try the effect of crude liver extract by the mouth. From this viewpoint exhepa was given in a dosage equivalent to 1½ lbs. of fresh liver daily commencing 18/10/38 and, at the suggestion of Dr. UNGLEY, liveroid (1½ ounces daily) was administered as well. Iron medication was continued. A dramatic response followed (Graph I). Fever disappeared on 27/10/38, the cardiac condition improved rapidly, and the reticulocytes rose to 13 per cent by the eleventh day and reached a maximum of 20·2 per cent. on the twenty-third day after commencement of oral liver therapy. Next day the M.C.V. showed a temporary increase to 100 c.μ. which

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* Reticulogen (Eli Lilly) is stated to contain in purified form the active anti-pernicious anaemia principle of liver in such concentration that an injection of 1 c.c. is comparable in haemopoietic effect to 13 to 20 lb. of fresh liver given by the mouth.
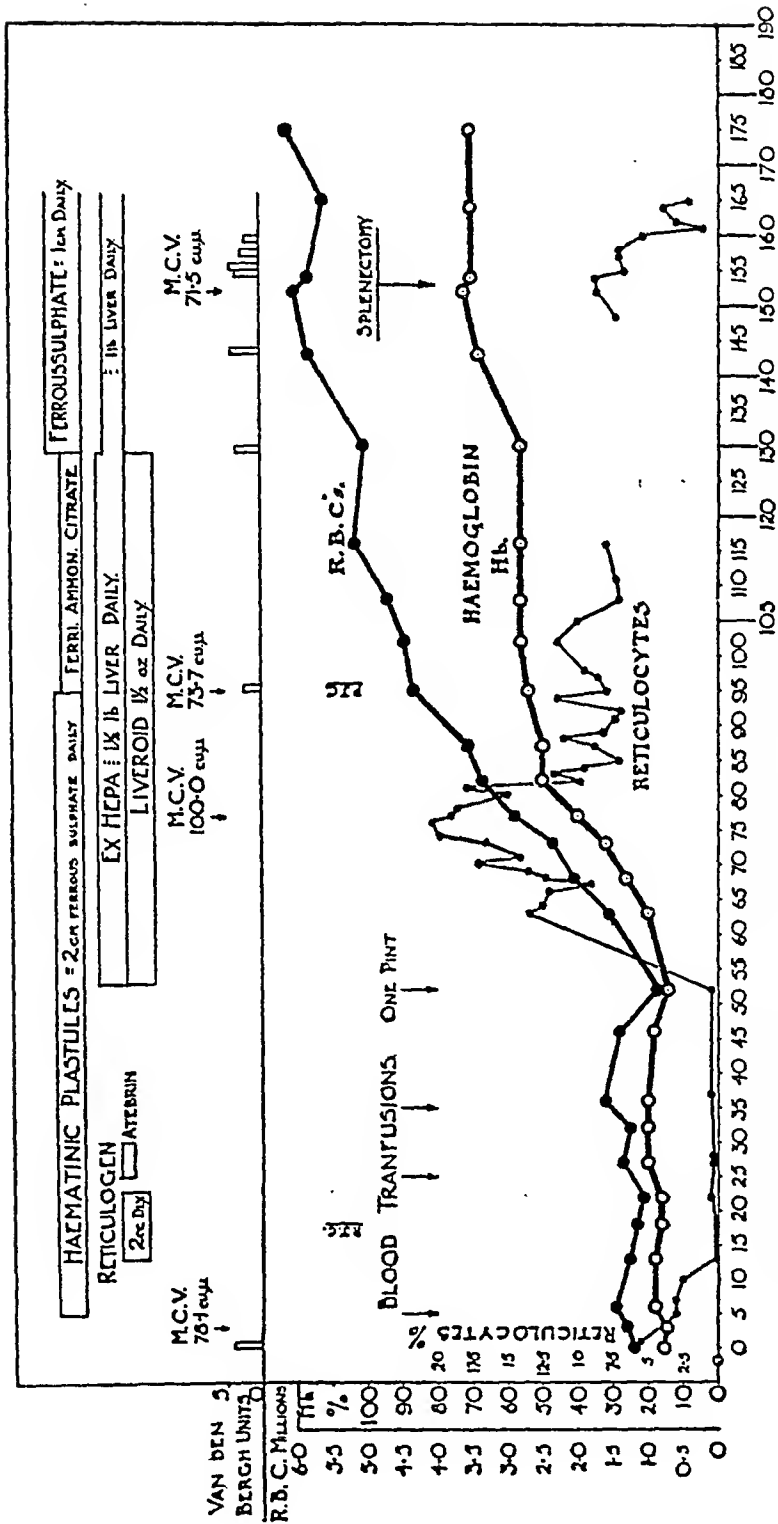
GRAPH I.—Haematological response following combined oral liver extract and iron therapy.
(Iron medication, both alone and combined with reticulogen and blood transfusions had previously failed.)

was directly related to maximal reticulocytosis ; by 29/11/38, when the reticulo-cytes had fallen to a lower level, the M.C.V. = 73·7 c.μ. On 11/11/38, R.B.C.s = 2,930,000 per c.mm. ; haemoglobin = 40 per cent. ; colour index = 0·7. Target cells and numerous nucleated red cells were present in films.

As seen in Graph I, the haemopoietic response continued to be satisfactory, though the haemoglobin production lagged behind that of the erythrocytes. On 25/1/39, the R.B.C.s = 6,010,000 per c.mm. and the haemoglobin 72 per cent. ; colour index = 0·6.

Persistence of Blood Destruction.—Throughout this period, and despite the great increase in the erythrocytes and haemoglobin, reticulocytosis persisted and evidences of blood destruction continued. Thus the haemobilirubin content of the blood varied from 2·5 to 3·5 van den Bergh units, and methaemalbumin was constantly demonstrable in a depth of serum varying from 2 to 4 cm. Schumm's ammonium sulphide haemochromogen test was invariably positive. Some time previously, Dr. JANET VAUGHAN had carried out an analysis of the stools for urobilinogen (stercobilinogen) over a period of 4 days. The daily excretion was 600 mg.—a very high figure of the order of that found in acholuric jaundice and pernicious anaemia and indicating a great excess of haemolysis. This finding was in marked contrast to the urinary excretion of urobilinogen which was not significantly increased. Its absence from the urine may be regarded as evidence of normal liver function. Both the intravenous galactose and the levulose tolerance tests for liver function were also normal.

It appeared possible that the spleen might be directly or indirectly responsible for the abnormal destruction of red cells and a surgical opinion was obtained on the practicability of splenectomy.

III.—SPLENECTOMY AND ITS EFFECT ON THE ANAEMIA.

On 26/1/39, Mr. A. H. MCINDOE performed a laparotomy. After ligating three enormous branches of the splenic vein and the splenic artery and separating some posterior adhesions, the greatly enlarged spleen was cleanly removed. Neither the splenic vein nor its branches were thrombosed. A spleniculus (3″ × 1″), located in the omentum above the pancreas was left *in situ*. The gall bladder was normal, the liver was not enlarged and presented no suggestion whatever of fibrosis ; the pancreas and other abdominal viscera appeared normal.

PATHOLOGICAL REPORT.

On removing the artery forceps from the dilated branches of the splenic veins much blood escaped, and the organ became flabby and collapsed. The spleen was firm, greatly enlarged and weighed 72 ounces. Its capsule was thickened, slaty-blue in colour and smooth except for a few adhesions and one depressed sclerosed scar. On section the cut surface was maroon-coloured ; it presented a tessellated appearance due to trabeculation ; Malpighian bodies were

not visible. A wedge-shaped area of sclerosed tissue, due to old infarction, corresponded with the scar on the capsule referred to above.

Professor G. R. CAMERON made a detailed histological investigation of the spleen and summarized his findings as follows : (1) thickening of the capsule and trábeculae with general diffuse increase of reticulum and collagen in the pulp ; (2) " Fibro-adenie " from marked dilatation of the pulp sinuses ; (3) inactivity of the pulp and lymphoid tissues as a whole ; (4) small areas of pulp hyperplasia ; (5) haemosiderosis with sclerotic nodules. There was very little R.E. cell activity and the findings were identical with the original histological picture described by BANTI in the disease which bears his name.

<div align="center">POST-OPERATIVE PROGRESS.</div>

The patient made a rapid and uninterrupted recovery and became fitter than he had ever remembered. He remained in hospital under observation until 6/5/39, and subsequently finished his course of studies and is now at work. Splenectomy, however, only temporarily controlled the haemolytic process with its consequent tendency to anaemia, and periodically the patient has required iron and liver medication to maintain the haemoglobin and red blood corpuscles at a reasonable level. The most striking changes now evident in the blood picture are the high percentage of target cells, the megalocytosis associated with an abnormally small mean corpuscular thickness and a marked decrease in corpuscular fragility to hypotonic saline solutions. The actual effect of operation may best be considered under several different headings.

(1) *Red Blood Corpuscles and Haemoglobin, etc.*—During his stay of 18 weeks in hospital after operation the R.B.C.s varied from 5,050,000 to 6,080,000 per c.mm., the haemoglobin from 56 to 70 per cent., the colour index from 0·55 to 0·7 and the reticulocytes from 1·2 to 8·8 per cent. On 5/5/39, the day before leaving hospital, the R.B.C.s = 5,440,000 per c.mm., the haemoglobin = 70 per cent. and the colour index = 0·65.

Since leaving hospital, treatment with liver extract and iron has only been periodically taken and in the intervals between treatment anaemia has developed. Till the end of 1939, the R.B.C.'s varied from 3,150,000 to 4,380,000 per c.mm., the haemoglobin from 46 to 60 per cent. and the colour index from 0·7 to 0·76. On two occasions the reticulocytes were counted and found to be 7·8 and 7 per cent. respectively. The peripheral blood film showed all the changes previously described and, in addition, an increase in the number of nucleated red cells (normoblasts and erythroblasts) and large numbers of megalocytes and target cells. On 21/11/39 target cells constituted 47·5 per cent. of the erythrocytes.

(2) *Corpuscular Fragility.*—The detailed results of changes in corpuscular fragility to hypotonic saline (Dacie-Vaughan technique, 1938) may be studied in Graph 2. On admission the observed mean corpuscular fragility (M.C.F.) was normal despite the grave nature of the anaemia. No target cells were then

present. On 11/11/38, after treatment had commenced and the count had risen to 2,930,000 per c.mm. and the haemoglobin to 40 per cent., the fragility had decreased somewhat, *i.e.* the observed M.C.F. = 0·350 per cent. NaCl. Target cells were now appearing in small numbers. Just prior to operation on 25/1/39 a further reduction of the observed M.C.F. to 0·313 per cent. NaCl was recorded. Some 26 days after operation it had further decreased, the observed M.C.F. being 0·18 per cent. NaCl. A similar finding was recorded 10 months later, *i.e.* on 21/11/39, when the observed M.C.F. = 0·186 per cent. NaCl. Target cells at this time were very numerous, *i.e.* 47·5 per cent. When anaemia was present no correction was made. According to DACIE and VAUGHAN (1938), however, the more anaemic the blood the less fragile the cells become, but in this case fragility decreased despite improvement in the anaemia following therapy. In the same way, though the M.C.T. : M.C.D. ratio decreased after operation, the fragility also decreased. These apparent anomalies are explained by the appearance of target cells in small numbers following medical treatment and in much larger numbers after splenectomy.

(3) *Price-Jones Curves, M.C.V., etc.*—Data relating to the Price-Jones curves, the M.C.V., M.C.T. and the M.C.T. : M.C.D. ratio are given in Table I (p. 175).

It will be seen that the Price-Jones curve taken on 13/9/38 before effective treatment had been initiated revealed a normocytic type of anaemia with a microcytosis of 2·0 per cent. and an M.D. of 7·113μ. The M.C.V., though low, fell within normal limits.

The curve made on 11/11/38 was taken some 24 days after effective treatment had started at a time when the sustained reticulocyte response (18·8 per cent.) still approximated to the maximum. On this occasion the M.C.V. had risen to 100 c.μ, the M.D. to 7·509μ and the megalocytes to 12 per cent. ; the change in the curve, however, was really due to large-sized reticulocytes and not to ordinary megalocytes. Later, on 29/11/38, when the reticulocyte count had decreased, the megalocytosis had completely disappeared and been replaced by a microcytosis of 5·6 per cent. ; the M.D. had also decreased to 6·933μ and the M.C.V. to 73·7 c.μ. In both these curves the M.C.T. fell within normal limits and was 2·26 and 1·95μ respectively.

The curve taken on 8/2/39, some 13 days after operation, showed a wide base (v = 14·3 per cent.) with a microcytosis of 8·6 per cent. and a megalocytosis of 3·4 per cent. The M.D. = 7·20μ ; the M.C.V. was very low, *i.e.* 68·6 c.μ, and the M.C.T. only 1·68μ. Finally, the curve taken on 21/11/39, almost 10 months after operation, showed extreme variability (v = 14·53 per cent.) and megalocytosis (59 per cent.). The M.D. = 8·924μ, the M.C.T. = 1·37μ and the M.C.V. = 85·7 c.μ. Target cells constituted 47·5 per cent. of the erythrocytes in blood films on this date.

(4) *The Methaemalbumin and Bilirubin Content of the Blood.*—It is interesting to note that blood taken from the splenic vein at operation immediately after the
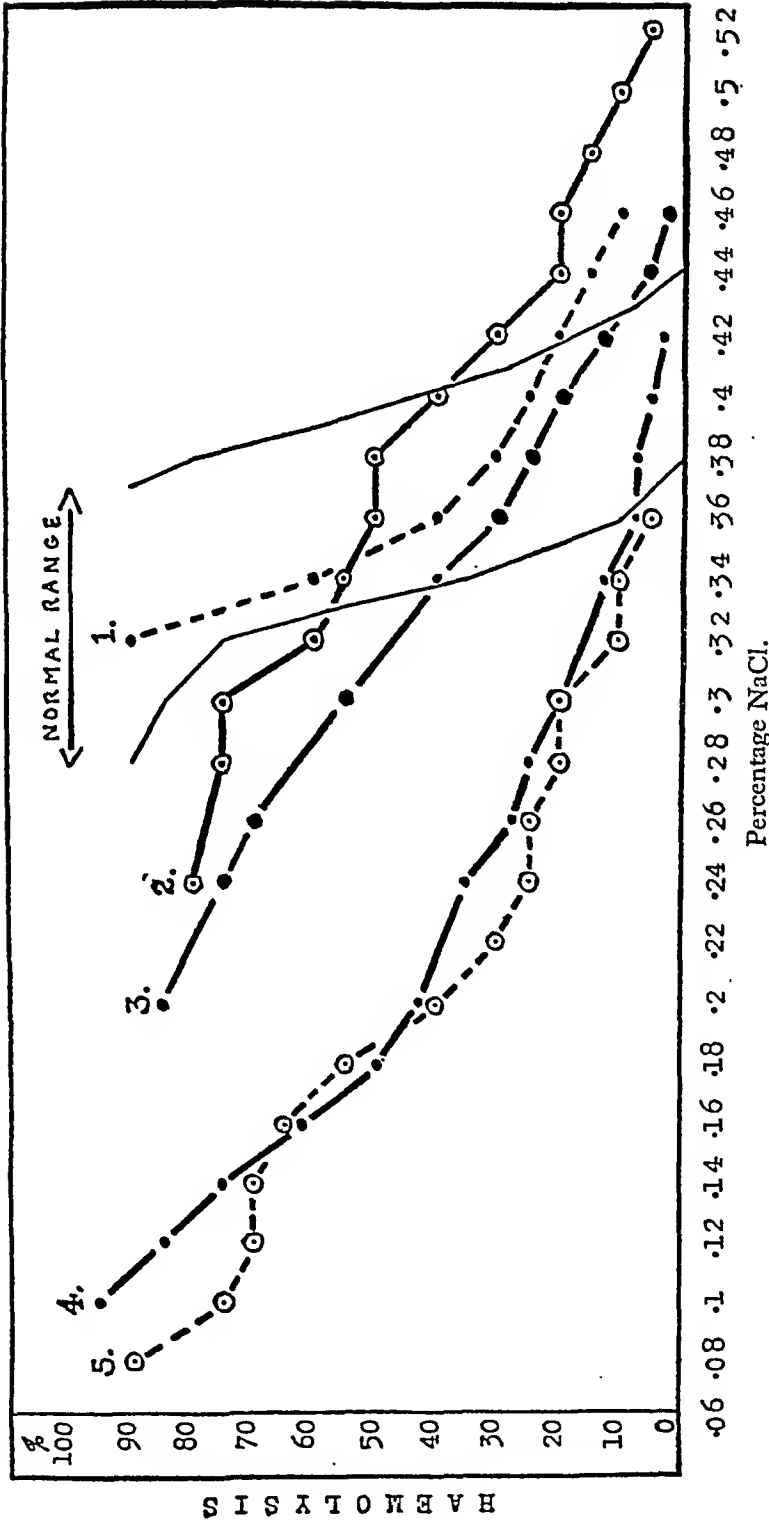
GRAPH II.—The effect on red cell fragility of anti-anaemic treatment and splenectomy.

1. M.C.F. = 0·37 per cent. NaCl (29.8.38)—before treatment.
2. M.C.F. = 0·35 per cent. NaCl (11.11.38)—during anti-anaemic treatment.
3. M.C.F. = 0·313 per cent. NaCl (25.1.39)—after anti-anaemic treatment.
4. M.C.F. = 0·18 per cent. NaCl (21.2.39)—after splenectomy.
5. M.C.F. = 0·186 per cent. NaCl (21.11.39)—after splenectomy.
                                    Splenectomy 26.1.39.

spleen was removed, contained the same concentration of methaemalbumin as the peripheral blood, *i.e.* the α band was just visible in a layer of plasma 2·0 cm. thick. The finding of 7·0 units of haemobilirubin in the splenic blood was considerably higher than the maximum value of 4·0 units recorded in the peripheral blood at any time during his illness. This was in accord with the findings of VAN DEN BERGH and SNAPPER (1915) in pernicious anaemia, where five out of six patients showed higher bilirubin values in the splenic blood collected at operation than in the peripheral blood.

The immediate effect of splenectomy on the bilirubin and methaemalbumin content of the peripheral blood is of interest. On the 2nd and 3rd day following operation the haemobilirubin equalled 3·5 and 4·0 units, by the 5th day it had decreased to 2·5 units, by the 6th day to 2·0 units, which was also the value recorded on the 14th day ; 2·5 units were present on the 27th day and 3 units on the 49th day following operation.

The α band of methaemalbumin was just visible spectroscopically in a thickness of 2 cm. of plasma examined in HARRISON's apparatus (1938) on the 2nd day, in a thickness of 3·5 cm. on the 3rd day, and was no longer demonstrable even in a layer of 11 cm. on the 4th, 5th, 6th and 14th days following operation. Schumm's test was also negative during this period. No further examination was made until the 32nd day by which time methaemalbumin had re-appeared and was visible spectroscopically in a layer of plasma of 3·5 cm. thickness. Some 9 days later, *i.e.* 41 days after operation, it had reached a maximum concentration observed, *i.e.* visibility in a layer of plasma of 0·5 cm. thickness. Schumm's test was strongly positive. Since then the haemobilirubin values have varied from 2·0 to 4·5 van den Bergh units, while the thickness of plasma through which the α band of methaemalbumin was just visible varied from 1·5 to 3·5 cm.

On three different occasions methaemalbumin was concentrated in the albumin fraction of this patient's serum free from all traces of haemoglobin. It invariably gave the typical spectrum and chemical reactions characteristic of this pigment.

Following splenectomy there was a temporary phase in which methaemalbumin was not produced in demonstrable quantity, indicating that during this period intravascular destruction of blood was reduced to a minimum. The significance of this observation will be discussed later.

COMMENT.

An outstanding feature in this case from the aetiological viewpoint is the prolonged history of recurrent malarial fever, anaemia and splenomegaly throughout childhood up to the age of 14 years.

Diagnosis and Treatment.—Pathological examination of the spleen by Professor G. R. CAMERON revealed a histological picture closely resembling that originally described by BANTI (1894) in his Italian cases. OSLER (1900 and 1902) drew attention to the history of chronic malaria infection in childhood in certain

of his cases of splenic anaemia and both syphilis and malaria are recognized as causing a splenomegaly which has to be differentiated from so-called Banti's disease.   Hepatic cirrhosis and haematemesis were both absent in this patient and if the diagnosis of Banti's disease were accepted, the patient would have to be regarded as being in the first stage of the disease.

The profound degree of anaemia, however, its haemolytic character and the presence of a leucocytosis are not in accord with such a diagnosis.   The fact that the anaemia which was characterized by marked hypochromia and a tendency to microcytosis, responded to oral crude liver extract therapy and iron, but failed to do so with iron or iron combined with injections of the refined liver extract, reticulogen, was a peculiar feature of the case and one which is difficult to explain in terms of present knowledge.   It suggests that the persistent erythropoiesis necessitated by the continued destruction of red cells in the blood stream had led to a secondary deficiency in some haemopoietic substance or substances necessary for blood regeneration which differed from the anti-pernicious anaemia principle contained in refined reticulogen, but which was present in crude liver. Despite splenectomy and the restoration of the corpuscles to over 5,000,000 per c.mm. by prolonged treatment, blood destruction continued ; an average dietary was evidently not rich enough in various haemopoietic substances to maintain the corpuscles and haemoglobin at a satisfactory level under these circumstances, and periodic treatments with crude liver extract and iron proved necessary.

Target Cell Development and its Implications.—As far as the post-operative blood picture is concerned, the persistent reticulocytosis and the large number of normoblasts and erythroblasts in the peripheral blood were a direct result of the continued erythroblastic response of the bone marrow consequent on persistent blood destruction.   Sternal puncture showed an erythroblastic marrow in a state of great activity.

The post-operative megalocytic anaemia was associated with (1) a normal M.C.V., (2) an abnormally small M.C.T., (3) a marked decrease in corpuscular fragility to hypotonic saline.   Target cells constituted 47·5 per cent. of the erythrocytes on films taken on 21/11/39 and it is obvious that these cells with their large diameter and decreased corpuscular thickness account for the anomaly of a profound megalocytosis associated with a normal mean cell volume.   These features along with decreased erythrocytic fragility to hypotonic saline are a known characteristic of the target cells.   Further, BARRETT (1938) in a very detailed study of these cells found that the conditions favouring their development were hyperbilirubinaemia and splenectomy.   Both these factors were present in this case.

Mechanism of the Haemolysis.—The mechanism of the haemolysis is a matter of speculation and efforts to demonstrate a haemolysin in the blood before and after splenectomy failed.   Compensating hypertrophy of R.E. in other parts

of the body, including the spleniculus left behind at operation, would naturally follow splenectomy and if its function—like that of the spleen—was pathologically perverted, the re-appearance of the haemolytic agent in the blood stream and the re-establishment of intravascular haemolysis would follow. This is suggested as the best working hypothesis to explain the temporary disappearance of methaemalbumin following splenectomy and its re-appearance later in the peripheral blood.

Perversion of R.E. Cell Activity as a Result of Chronic Malaria.—It is well recognized that chronic malaria induces considerable hypertrophy of the R.E. system in general and that of the spleen in particular, and that acquired immunity to malaria depends on this fact.

Unpublished observations indicate that the prolonged chronic irritation induced by recurrent malaria on hypertrophied endothelium may lead to a perversion of normal endothelial cell activity and function which may result in (1) a grossly abnormal R.E. cell hypertrophy, which in extreme cases may simulate monocytic leukaemia from a pathological though not a haematological viewpoint, (2) a pathological increase in the normal phagocytic function of the R.E. leading to excessive destruction of erythrocytes such as occurs in nutritional macrocytic anaemia associated with chronic malaria in Macedonia, and which has been described by FAIRLEY, FOY, BROMFIELD and KONDI (1938), and (3) an escape into the blood stream from pathological R.E. of the intracellular lytic enzyme or lysin normally responsible for the destruction of effete or damaged phagocytosed red cells. The sudden liberation into the circulating blood of large amounts of such a lytic enzyme would explain the phenomena associated with blackwater fever, while a chronic overflow in small quantity would account for a haemolytic anaemia of the type described in this communication. With intracellular lytic enzyme one might anticipate that complement would not participate in the haemolytic reaction, and that as the lytic agent was liberated from the endothelial cells in the internal organs, it would become immediately fixed to the corpuscles and be no longer demonstrable in the peripheral blood.

In this connection it may be remembered that phosphatidases are responsible for venom haemolysis. The haemolytic activity of the Australian snake venoms is directly related to their lecithinase content, and KELLAWAY (1939) has recently suggested that the prehaemolytic swelling of the corpuscle observed in venom haemolysis may be explained by the formation of lyso-lecithin which gives an expanded, fragile and more permeable film because the area per hydrocarbon chain is nearly double that in a lecithin film in the same state of compression.

Space does not permit a more detailed discussion on this subject, which will form the basis of another communication.

Summary and Conclusions.

(1) A peculiar haemolytic hypochromic anaemia associated with post-malarial spenomegaly of Banti's type is recorded for the first time.

(2) An unexpected feature was the effectiveness of orally administered crude liver extract preparations and iron in inducing a satisfactory haemato-poietic response, following the previous failure of injections of a refined liver extract (reticulogen) combined with iron medication and repeated blood transfusion.

(3) Following splenectomy, a haemolytic megalocytic erythroblastic anaemia developed associated with a great increase in target cells and decreased fragility of the corpuscles to hypotonic saline solution.

(4) Methaemalbuminaemia temporarily disappeared following splenectomy but reappeared later.

(5) The escape of an intracellular lytic enzyme into the blood stream from pathological reticulo-endothelium following prolonged chronic malarial infection is suggested as a possible basis for the condition.

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RECURRENT BLACKWATER FEVER INDUCED BY QUININE.

BY

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The close correlation between the administration of quinine to malarial patients and the onset of blackwater fever has long been recognized, but it is not often that a malarial patient is found in whom the attacks of haemoglobinuria induced by quinine are sufficiently mild and sufficiently frequent to enable an investigation to be undertaken both during and between the paroxysms of intravascular haemolysis which constitute the fundamental basis of blackwater fever.

All the features regarded as essential in the diagnosis of blackwater fever were fulfilled in this case. The patient had lived in parts of India where blackwater fever is known to occur. When she was admitted to hospital with fever and splenomegaly, malarial parasites were found in her blood. Haemoglobinuria followed the administration of quinine but skin tests failed to show evidence of dermal hypersensitivity to quinine and there was no reason for regarding the case as one of quinine haemoglobinuria rather than of blackwater fever.

CASE.

The patient, a female aged 49 years, was admitted to the Hospital for Tropical Diseases on 2/6/39 with fever which had developed 3 days previously; benign tertian parasites and crescents of malignant tertian malaria were found in blood smears the next day.

History.—The patient stated she had lived in India and Assam for 20 years. She had suffered from recurrent attacks of malaria from 1921 to 1930, but subsequently had been entirely free from fever until 3 days before admission to hospital; then she was seized with a rigor and fever followed by severe sweating, since when rigors had occurred at 24-hourly intervals. She also complained of severe headache and cramp in the legs. There was amenorrhoea of 7 months' duration associated with the climacteric.

Physical Examination.—Patient well nourished; tongue coated; icteric tinge of skin; no obvious anaemia; the spleen was enlarged; scoliosis present.

Laboratory Findings.—Blood smears on 3/6/39 showed benign tertian parasites and subtertian crescents. R.B.C.s = 4,400,000 per c.mm.; haemoglobin = 86 per cent.; colour index = 1·0. The urine contained only a trace of urobilin.

First Attack of Blackwater Fever.

The patient was given quinine bihydrochloride, 10 grains t.d.s. and plasmoquine 0·02 gramme t.d.s. commencing on 3/6/39. This was continued on 4/6/39, but at 6 p.m. the patient developed mild blackwater. She had a temperature of 103° F., complained of feeling very hot, became drowsy and sweated profusely. The urine was then dark brown in colour and contained oxyhaemoglobin and some methaemoglobin. Anti-malarial treatment was stopped and potassium citrate and sodium bicarbonate were given to alkalinize the urine.

Blood counts next day (5/6/39) showed R.B.C.s = 3,600,000 per c.mm. and haemoglobin = 76 per cent. (Haldane)—a loss of 800,000 corpuscles per c.mm. and of 10 per cent. haemoglobin as a result of intravascular haemolysis. The van den Bergh reaction gave a positive indirect reaction (1·5 units) and a negative direct reaction. Methaemalbumin was demonstrable spectroscopically but only in very thick layers of plasma. No parasites were found in blood films.

Second Attack of Blackwater Fever.

After an interval of 2 days, during which the temperature was normal, quinine bihydrochloride was given in progressively increasing dosage as follows:—

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\begin{array}{llll}
7/6/39—\tfrac{1}{2}\ \text{grain} & \times\ 3 = & \text{total}\ 1\tfrac{1}{2}\ \text{grains} \\
8/6/39—1\quad ,, & \times\ 3 = & ,,\quad 3 \quad ,, \\
9/6/39—2\quad ,, & \times\ 3 = & ,,\quad 6 \quad ,, \\
10/6/39—4\quad ,, & \times\ 3 = & ,,\quad 12 \quad ,, \\
11/6/39—7\tfrac{1}{2}\ ,, & \times\ 3 = & ,,\quad 22\tfrac{1}{2}\ ,, \\
12/6/39—10\quad ,, & \times\ 3 = & ,,\quad 30 \quad ,, \\
13/6/39—10\quad ,, & \times\ 3 = & ,,\quad 30 \quad ,,
\end{array}
$$

From 9/6/39 potassium citrate and sodium bicarbonate were given to alkalinize the urine.
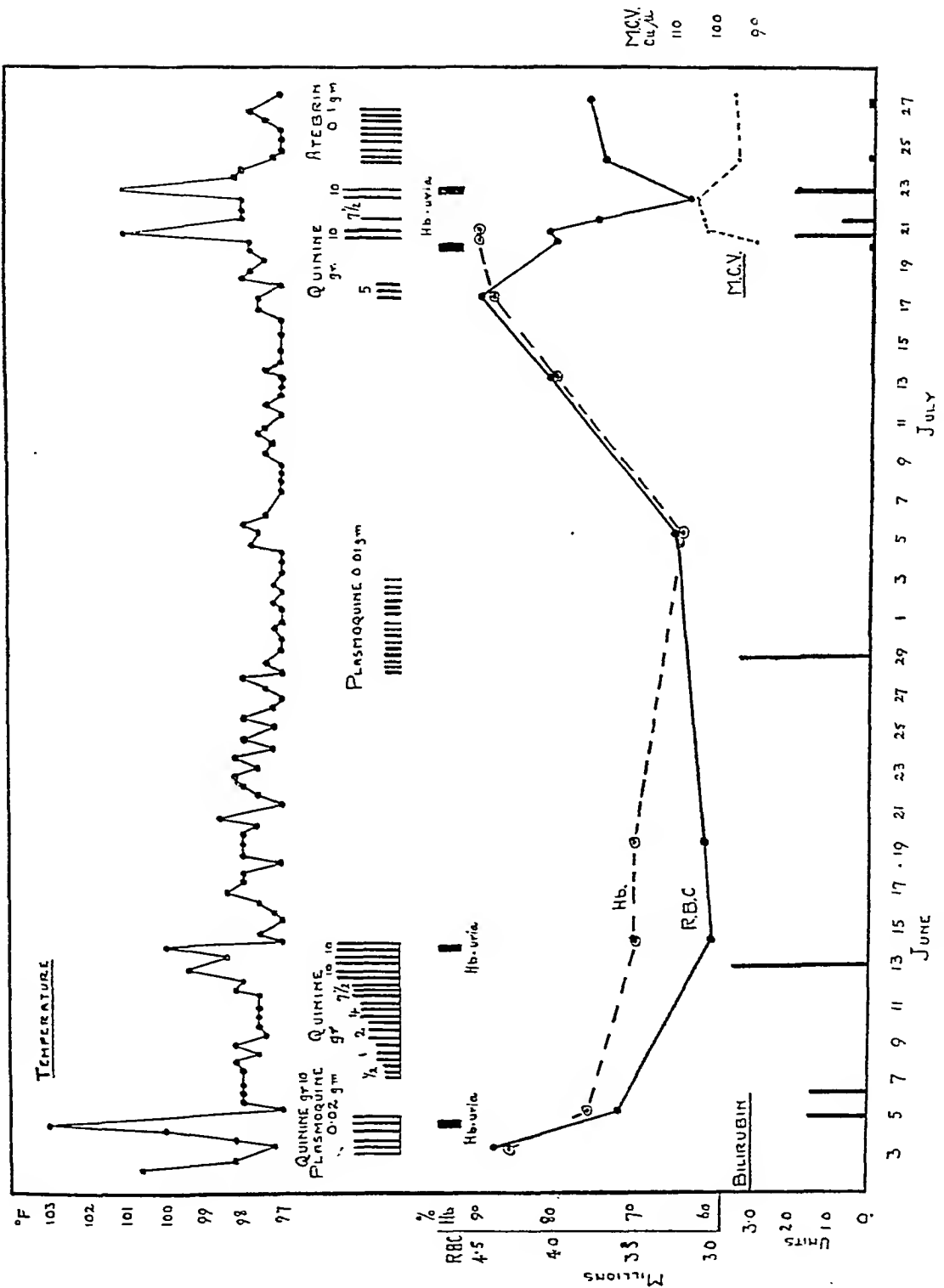
Data in recurrent attacks of blackwater fever induced by quinine.

The patient complained of feeling "seedy" on 10/6/39 and developed an evening temperature of 100° F. on 11/6/39. On 12/6/39 there were no parasites in the blood, but the temperature rose to 101·4° F. about 10 p.m. On 13/6/39 at 7 p.m. she had transient haemoglobinuria and the evening temperature was 100·8° F. The haemobilirubin had risen to 3·5 van den Bergh units (indirect reaction). By 9 p.m. the urine was clear of blood pigment. Anti-malarial treatment was stopped. Next day (14/6/39) the patient had a slight headache and looked icteric. *Blood examination* showed: R.B.C.s = 3,000,000 per c.mm.; haemoglobin = 70 per cent. (Haldane); colour index = 1·2: aniso-cytosis and poikilocytosis present: no parasites seen; leucocytes = 3,900 per c.mm.; neutrophils = 43 per cent.; lymphocytes = 43 per cent.; monocytes = 12 per cent.; eosinophils = 2 per cent.

Treatment for Anaemia.—The temperature had returned to normal on 14/6/39 and remained so for 38 days. On 19/6/39 the R.B.C.s = 3,040,000 per c.mm. and haemoglobin 70 per cent. (Haldane), and haematinic plastules co. in a dosage of 2 plastules t.d.s. were given from 23/6/39 until 14/7/39. Anti-malarial treatment was recommenced on 28/6/39 when plasmoquine 0·01 gramme t.d.s., was administered for 5 days without haemoglobinuria developing. On 5/7/39, R.B.C.s=3,250,000 per c.mm.; haemoglobin=64 per cent. (Haldane); colour index = 1·0, and from 8/7/39 to 14/7/39, 4 c.c. of campolon were injected daily. By 13/7/39 the anaemia had definitely decreased: R.B.C.s = 4,060,000 per c.mm.: haemoglobin = 80 per cent. (Haldane); colour index = 1·0.

Third and Fourth Attacks of Blackwater Fever.

It was decided to ascertain whether quinine could now be given without inducing haemoglobinuria; as a preliminary measure an alkaline-citrate mixture was administered on 16/7/39 to alkalinize the urine. Next day (17/7/39) R.B.C.s = 4,500,000 per c.mm.; haemoglobin = 88 per cent. (Haldane); colour index = 1·0: quinine bihydrochloride, 5 grains, was given three times without apparent effect except that the patient did not feel quite as well as usual. Schumm's test for haematin, which was negative before, became positive the day after quinine was administered. No quinine was given on 18/7/39 or 19/7/39.

20/7/39.—Quinine bihydrochloride, 10 grains, was given at 11 a.m. and 2 p.m. At 3 p.m. the patient complained of deafness and at 5.30 p.m. passed dark red urine, neutral in reaction, and containing oxyhaemoglobin, excess of urobilinogen and a trace of methaemoglobin. By 8.30 p.m. the temperature was 101·4° F., the patient complained of shivery feelings and sweating, and the spleen was palpable 1 inch below the costal margin. The 9.30 p.m. specimen of urine also contained oxyhaemoglobin and an excess of urobilinogen, but no methaemoglobin.

The blood was first examined at 11.30 a.m., just half-an-hour after the administration of quinine, and again at 8.30 p.m. several hours after the onset of haemoglobinuria. The haematological findings on this day and during the next week are epitomized in Table I. Plasma collected at 11.30 a.m. prior to the haemoglobinuria gave a positive Schumm's test for methaemalbumin which, however, was not present in sufficient concentration to be demonstrable spectroscopically; there was no excess of haemobilirubin. The evening specimen of plasma collected at 8.30 showed definite haemoglobinaemia and gave a strongly positive Schumm's test, while spectroscopic examination through a layer of 5 c.m. thickness showed methaemalbumin; the haemobilirubin had risen to 3·0 van den Bergh units. Changes in the red cells were of equal interest (Table I). Comparing morning and evening specimens, the M.C.V. (mean corpuscular volume) had risen from 89·8 to 102·6 c.μ; the M.D. (mean diameter) from 6·86 to 6·942μ; and the M.C.T. (mean corpuscular thickness) from 2·43 to 2·71μ. This indicated that during the phase of intravascular haemolysis there was definite swelling of the corpuscles with increase in thickness, i.e., spherocytosis. At 11.30 a.m. the M.C.F. (mean corpuscular fragility) determined by the Dacie-Vaughan technique was 0·38 per cent. NaCl; by 8.30 p.m. the M.C.F. was 0·405 per cent. NaCl, indicating an increased fragility.*

21/7/39.—The first specimen passed at 1.50 a.m. had cleared and was free from blood pigment. The patient felt somewhat nauseated, but had no temperature and the spleen was still palpable. Quinine bihydrochloride, 7½ grains, was injected intramuscularly at 9.30 a.m. Haematological findings in the blood collected at 12.45 p.m. are shown in Table I. Though the M.C.T. had decreased, the M.D. had somewhat increased and the M.C.V. remained at an abnormally high level of 102·9 c.μ; The M.C.F. had slightly decreased to 0·395 per cent. NaCl., Schumm's test was positive, the haemobilirubin had decreased to 0·8 units (indirect), while methaemalbumin was just demonstrable spectroscopically in a layer of plasma 10 cm. thick.

22/7/39.—Quinine bihydrochloride, 10 grains, was given by the mouth, at 4 a.m. and 12 noon. By 4 p.m. the patient was complaining of slight deafness and shivering, and passed 1½ ounces of dark brown urine containing blood pigment: temperature = 101° F. The next specimen of urine at 5.45 p.m. was clear. At 7 p.m. temperature = 101·8° F.: the patient was feeling better but had vomited once; the spleen was palpable about ½ inch below the costal margin. Blood collected at this time, i.e., 7 p.m., showed a further fall in the number of R.B.C.s (Table I). There was a decrease in the M.D. to 6·87μ, and increase in the M.C.V. to 105·4 c.μ, and an increase in the M.C.T. to 2·84μ: spherocytosis was therefore marked. The M.C.F. had also increased considerably to 0·43 per cent. NaCl. A definite haemoglobinaemia was present; the

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*The calculated normal M.C.F. according to DACIE and VAUGHAN is 0.366 per cent. NaCl, the range, in health, being 0.334-0.398 per cent. NaCl.

plasma gave a strongly positive Schumm's test; methaemalbumin was visible spectroscopically in a layer of plasma 5·5 cm. thick, while the haemobilirubin had risen to 3·0 units.

These findings indicated a persistence of corpuscular spherocytosis associated with intravascular haemolysis and suggested that the corpuscular fragility to hypotonic saline had also increased. Further administration of quinine was stopped.

24/7/39.—Skin tests for hypersensitivity to solutions of quinine bihydrochloride in strengths of 1/10, 1/100, 1/1,000 and 1/10,000 yielded entirely negative results. The haemobilirubin had fallen to 0·2 units (indirect) and methaemalbumin had disappeared. The red cell count showed some improvement, but spherocytosis was still demonstrable on this date as well as on 27/11/39 as indicated respectively by M.C.T. of 2·675μ and 2·69μ (Table I).

No further fever occurred and the patient, who was given 0·1 gramme of atebrin t.d.s. for the next 3 days without any deleterious effect, was discharged on 27/7/39.

Subsequent Progress.—The patient was given another complete course of atebrin in October (0·1 gramme t.d.s. for 5 days). Up to December, when she sailed for India, no fever, haemoglobinuria or other untoward symptoms developed. When seen on 27/11/39 she appeared to be in excellent health. The spleen was not palpable and the blood contained no malarial parasites. R.B.C.s = 4,960,000 per c.mm.; haemoglobin = 92 per cent. (Haldane); colour index = 0·9. The Price-Jones curve was normal in all respects (Table I), the M.C.V. = 84·6 c.μ, and the M.C.T. = 2·07μ. The only abnormality found at this time was an increased corpuscular fragility to hypotonic saline solution, the M.C.F. being 0·41 per cent. NaCl.

After her arrival in India at the end of February, 1940, she was given a course of quinine 10 grains thrice daily for five days during which time the urine remained free from any trace of albumin or haemoglobin. She has since been taking a daily dose of quinine 6 grains and at the end of April was reported to be looking and keeping very fit.

Discussion.

This case presents several features of unusual interest. Clinical and laboratory observations both indicate that the capacity of quinine to produce haemoglobinuria was directly or indirectly related to persisting malaria infection. For some 2 months after malaria parasites were found, during which period the spleen was demonstrably enlarged, the administration of quinine led to haemoglobinuria at a time when the patient presented no other evidence of idiosyncrasy to quinine, and dermal hypersensitivity to this drug could not be demonstrated. Subsequently, atebrin was given without any ill effects and several months later, when all evidence of previous malaria had disappeared,

quinine for 5 days in large amounts and in smaller doses over a longer period, entirely failed to produce haemoglobinuria.

On the biochemical side the transient attacks of haemoglobinuria were found to be associated with haemoglobinaemia, some increase in haemobilirubin and the presence of methaemalbumin. The latter pigment was not always demonstrable spectroscopically even when thick layers of plasma were examined, but Schumm's test was invariably positive and revealed its presence even when it was not in sufficient concentration to be visible spectroscopically.

TABLE.

HAEMATOLOGICAL DATA CONCERNING THE ERYTHROCYTES BEFORE, DURING AND AFTER THE INTRAVASCULAR HAEMOLYSIS IN BLACKWATER FEVER.

Date.	R.B.C.s (Millions).	M.D. (μ)	σ (μ)	v (Per cent.)	Megalocytes (Per cent.)	Microcytes (Per cent.)	M.C.V. (c.μ.)	M.C.T. (μ)	M.C.F. (Per cent. NaCl).
20.7.39* (11.30 a.m.)	4·01	6·86	0·413	6·02	0	0	89·8	2·43	0·38
20.7.39 (8.30 p.m.)	4·07	6·942	0·498	7·17	0	0	102·6	2·71	0·405
21.7.39 (12.45 p.m.)	3·74	7.263	0·486	6·67	0	0	102·9	2·48	0·395
22.7.39 (7 p.m.)	3·14	6·87	0·43	6·28	0	0	105·4	2·84	0·43
24.7.39 (3 p.m.)	3·71	6·703	0·419	6·25	0	3·0	94·3	2·675	—
27.7.39	3·83	6·718	0·459	6·83	0	2·4	95·3	2·69	—
27.11.39	4·96	7·213	0·541	7·5	0	0	84·6	2·07	0·41

*This specimen was collected within ½ hour of the commencement of quinine treatment.

The haematological data in the table show that swelling of the corpuscles accompanied the intravascular haemolysis responsible for the haemoglobinuria. This was indicated by the increased mean corpuscular volume (M.C.V.) which was observed over the period of 20/7/39 to 22/7/39, during which time the 3rd and 4th bouts of haemoglobinuria occurred. The mean corpuscular thickness was also increased throughout this period, the increase persisting for several days later. As the mean corpuscular diameter was not proportionately

increased, it follows that a condition of spherocytosis had been produced. The mean corpuscular fragility also showed an increase during this period compared with what was observed before the commencement of the haemoglobinuria. An increased corpuscular fragility to hypotonic saline solution was also observed some four months after the last attack of haemoglobinuria at a time when the patient had apparently recovered from malaria and the blood was otherwise normal: the interpretation of this finding is difficult and there was, unfortunately, no opportunity of repeating the observation as the patient shortly after sailed for India.

Prehaemolytic swelling of the corpuscle and the other haematological data reviewed above are compatible with the view that a lytic enzme or biological haemolysin is implicated in the haemolysis underlying in blackwater fever.   As already suggested by one of us (N.H.F.) the agent responsible may well prove to be the *intracellular* lytic enzyme normally employed in the destruction of phagocytosed red cells; perversion of function of a pathological reticulo-endothelial system hypertrophied and irritated as a result of chronic malarial infection, might culminate in a sudden liberation *of this lytic substance into the circulation with resulting intravascular haemo-* lysis and haemoglobinuria.

From this viewpoint, it is noteworthy that in the present case the spleen was demonstrably enlarged, and presumably the reticulo-endothelial system hypertrophied, throughout the period during which quinine therapy induced transient bouts of blackwater fever, whereas following effective treatment and disappearance of the splenomegaly, quinine administration was no longer followed by haemoglobinuria.

SUMMARY AND CONCLUSIONS.

(1) The laboratory and clinical data recorded refer to a malarial patient studied during recurrent attacks of mild blackwater fever induced by quinine.

(2) No evidence of dermal hypersensitivity to quinine was found.

(3) The capacity of quinine to produce blackwater fever appeared in this case to be directly or indirectly related to persisting malarial infection.

(4) Several months after apparent cure of the malaria, quinine therapy entirely failed to induce haemoglobinuria.

(5) During and following the bouts of haemoglobinuria, methaemalbumin was invariably demonstrated in the plasma either spectroscopically or by means of Schumm's test.

(6) Prehaemolytic swelling of the corpuscles associated with spherocytosis and probably increased fragility to hypotonic saline solution were observed.

(7) The findings suggest that the lytic agent acts directly on the circulating corpuscles, and are compatible with the view that a lytic enzyme or biological haemolysin was implicated.

A SIMPLE AND RAPID METHOD OF STAINING MALARIAL PARASITES IN THICK BLOOD SMEARS.

BY

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Dried smears of blood of a thickness not greater than about 50μ can be made transparent enough for microscopic examination by lysing the red cells and removing the haemoglobin. Conventional methods of staining malarial parasites in thick blood smears depend on this fact. The ideal method of staining would be one in which the red cells were alone affected during lysis of the blood, but in practice other cells—leucocytes and blood protozoa—are also damaged and we have to be content with a compromise giving maximal

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destruction of red cells with a minimal effect on the other cellular elements. Ordinarily this compromise is attained by drying the blood for several hours before staining. Prolonged drying produces a slight fixation of all the cells of the blood which, while enough to protect the white cells from gross damage during lysis, is not enough to hinder the solution of the erythrocytes. If the period of drying is reduced much below eight hours the white cells are liable to plasmolysis and may be so damaged during staining that they are scarcely recognizable. The difficulty of speeding up the standard Giemsa methods of staining thick blood smears is related mainly to these facts.

Methods of preserving the outlines of the leucocytes in Giemsa-stained thick smears and of rapidly staining malarial parasites without undue delay for drying the blood have recently been described by PAMPANA and by SIMONS.

PAMPANA (1938) draws attention to the fact that damage during staining to leucocytes and blood protozoa in thick blood smears may be reduced by diluting the stain with a phosphate buffer solution isotonic with blood serum —not, as is usual, with distilled water or with a hypotonic buffer. Fresh and dried blood, he points out, do not behave in like manner towards isotonic solutions, for the red cells in dried blood are lysed almost as readily by isotonic solutions as by distilled water. For routine Giemsa staining he advocates the dilution of the stain with a buffer solution of phosphate equimolecular with blood serum and of pH 7·2.*

SIMONS (1938) points out that leucocytes and blood protozoa are beautifully preserved in thick blood smears stained with a solution of methylene blue containing saponin and approximately isotonic with serum.† Lysis of the red cells and penetration of the stain are exceptionally rapid. By this method the stain is allowed to act on the dried smear for two minutes, a cover-slip is applied and after the excess of the stain has been cautiously blotted away the preparation is ready for microscopic examination. Smears may be stained within a few minutes of preparation without fear that they will detach from the slide or that the leucocytes or blood protozoa will be broken up. The main drawbacks of SIMONS' method of staining are that the smears are examined through a considerable depth of aqueous stain, and hence the blood cells or protozoa must be picked up at different focal levels: that they must be examined before the stain is dry: and that the stain, acting as a blue light filter, obscures the yellow colour of malarial pigment.

* Pampana's Isotonic Buffer Solution pH 7·2 :—

      NA_2HPO_4 14·8 grammes.   Distilled water 1,000 c.c.
      KH_2PO_4 5·3   ,,

† Simons' Stain :—

      Distilled water 300 c.c.   Sodium citrate 3·0 grammes.
      Methylene blue 0·6 grammes.   Saponin   2·0   ,,
      Sodium chloride 1·8   ,,   Formol 15%   12 c.c.

These observations seem to suggest that speed of staining and freedom from distortion of leucocytes and protozoa in thick blood smears may perhaps best be sought by the use of basic stains in isotonic solution. Various experiments based on this assumption have been made in this laboratory and a method has been devised whereby thick blood smears may be stained within 1 second sufficiently well for the accurate identification of malarial parasites. The method is briefly described in this preliminary communication as it may interest other workers concerned with the rapid diagnosis of malaria.

GENERAL PRINCIPLES OF THE METHOD.

The method depends essentially on the fact that certain basic dyes in aqueous solution are able to penetrate a depth of approaching 50μ of dried blood with extreme rapidity and are adsorbed by leucocytes and blood protozoa before lysis of the red cells is complete and before the red cell envelopes are stained so heavily that they obscure the microscopic field. The presence of free haemoglobin seems to retard the staining of the red cell residues which form the "background" of the stained thick smear and if the staining process is arrested at the moment when contrast is maximal, i.e., when leucocytes or protozoa have stained; but the red cell residues, protected by free haemoglobin, have not yet taken the stain—the leucocytes and protozoa are contrasted against a smooth pale yellow ground of residual haemoglobin. Contrast is optimal when the staining process is restricted to the pH range 6·6 to 7·0; and if, in addition, the stains are used in isotonic solution the outlines of leucocytes and blood protozoa are extraordinarily well preserved.

The essential conditions appear to be : —

i. The use of certain basic stains in isotonic solution to conserve the outlines of the leucocytes.

ii. Limitation of staining to about 1 second, at which time leucocytes and blood protozoa have taken the stain but the background is relatively unstained.

iii. The buffering of the stain to pH 6·6 to 7·0—the pH range at which contrast is maximal.

iv. The retention of some of the haemoglobin to provide a pale yellow background contrast to the stained parasites and to restrict the staining of the envelopes of the lysed red cells.

Various stains have been tested, singly or in combination. Those which have given the best results are methylene-blue, brilliant cresyl blue, mixtures of methylene blue and eosin and an aqueous extract of combined Giemsa powder: of these brilliant cresyl blue has been the most generally satisfactory.*

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* The staining of the malarial parasites with brilliant cresyl blue is not new. SYDENSTRICKER and VRYONIS (1935) for example, have described and illustrated the appearances of P. falciparum and P. vivax in fresh blood stained with brilliant cresyl blue in physiological saline. The method, however, is a standard method of vital staining and differs radically from that described in this paper.

PREPARATION OF STAIN.

The composition of the stain recommended is as follows: —

Brilliant cresyl blue	1·0 gramme
Disodium hydrogen phosphate (anhydrous) ...	1·0 gramme
Potassium dihydrogen phosphate (anhydrous)	1·25 gramme
Distilled water	100 c.c.

The phosphate salts are first dissolved. The solution is approximately isotonic with blood serum and is of pH 6·6. The stain is now added, solution being aided by grinding in a mortar. After filtration the stain is ready for use. Occasional filtration subsequently is desirable as a thin scum may form which is liable to deposit on the slides *

METHOD OF USE.

The blood smears should not be more than 50μ thick; if they are too thick there is too great a depth of residual haemoglobin. The thickness is about right if with the slide held near the face of a watch the hands can ·be dimly seen through the dried smear. The smears are rapidly dried by waving in the air or in a hot-air current and lightly heat-fixed by passing through a flame for about a second. · The slides should not be made so hot that they cannot be held against the back of the hand. To stain the smears the slides are dipped for 1 second into a jar containing the stain, immediately removed and rinsed for 5 seconds by waving *gently* in a vessel of clean tap water. They are now placed vertically on a rack to drain and dry and are then ready for microscopic examination.

During drying it will be noted that the haemoglobin continues to drain from the smear down the slide, leaving in the final dried smear four distinct zones depending on the amount of haemoglobin left behind.

A. A zone at the upper thin edge of the smear from which most of the haemoglobin has drained away and in which on microscopic examination the background is seen to be stained a pale blue grey. In this zone minute blood parasites, such as young *P. falciparum* rings, are best sought.

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* An alternative stain, giving rather less brilliant but otherwise similar staining, can be prepared from methylene blue and eosin in proportions as follows :—

Methylene blue 0·4 grammes, Eosin 0·1 gramme, Isotonic phosphate buffer 100 c.c.

The stains are independently dissolved—the methylene blue in 80 c.c. of the phosphate solution and the eosin in 20 c.c.—and mixed by the addition of the eosin solution to that of the methylene blue. After filtration the stain is ready for use. Subsequent filtration is essential whenever a scum appears on the surface of the stain.

B. An adjoining zone where there is sufficient residual haemoglobin to colour the background a smooth pale creamy yellow. In this zone colour contrast is maximal—vivid blue against pale yellow—and here most malarial parasites, excepting only small *P. falciparum* rings, are best defined.

C. A central zone where there is too much residual haemoglobin for clear definition and where only the leucocytes and larger blood protozoa are well seen.

D. A lower zone at the lower thin edge of the smear where leucocytes and the larger parasites are often beautifully contrasted against a smooth yellow ground.

The technique of staining and examination may be summarized as follows:—

1. Rapidly dry the smear and lightly fix in a flame.

2. Dip the smear into a jar of stain for 1 second.

3. Differentiate in clean tap water for 5 seconds.

4. Drain and dry by placing *vertically* against a staining rack.

5. Examine Zone A for *P. falciparum* rings and either of Zones B or D for other malarial parasites.

TABLE.

	Thin film diagnosis.	Wrong species diagnosis in " rapid " thick smear.	Species diagnosis uncertain or parasites not recognized in " rapid " thick smear.
P. falciparum	239	1	4
P. vivax ...	103	1	1
P. malariae	18	0	0
Mixed ...	23	0	13*

* One species recognized.

Using this technique and rapidly drying the stained smears in a current of hot air it has been found possible to diagnose malaria and to identify the species and phase of parasite with surprising accuracy within one and a half minutes of the first preparation of the smears.

Reliability.

Thick blood smears stained by this rapid method have now been compared with Giemsa-stained thin films in 383 cases of acute malaria. The accuracy of diagnosis from the rapidly stained films is indicated in the table above.

Diagnostic Comparison of Rapidly Stained Thick Smears with Stained Thin Films in 383 Cases of Acute Malaria.

Parasites were usually seen in the thick smears within the first few microscopic fields, sometimes when in the thin films they had been found only after a prolonged search.

In 250 cases the parasites found in the first ten microscopic fields were counted. The relative thick smear and thin film counts were as follows:—

Average number of parasites per 10 thin film fields   6·2
Average number of parasites per 10 thick smear fields   99·2

There was thus a sixteen-times concentration of parasites in the thick smears, a figure which, when familiarity with the appearances of the parasites in the thick smears has been acquired, is probably a fair indication of the relative speed of diagnosis.

MORPHOLOGY OF MALARIAL PARASITES STAINED BY THIS RAPID METHOD.

Malarial parasites in thick smears stained by this rapid method have a general resemblance to those seen in smears stained by standard Giemsa methods. As in Giemsa-stained smears the host cells have disappeared and the parasites are seen on a background derived from the lysed red cells. The main departures from the usual appearances in Giemsa-stained smears are that:—

(a) The cytoplasm tends to be more vividly stained.

(b) The chromatin is poorly stained and not differentiated in colour.

(c) Small " ring " forms tend to be pale and somewhat ill-defined.

(d) Nuclear and reticular débris from immature red cells is more deeply stained.

DESCRIPTION OF PLATE.

FIG. 1.—*P. falciparum* : " ring " forms in thick blood film stained for one second. The rings are pale and delicate ; the chromatin is less well differentiated than in Giemsa-stained films.

FIG. 2.—*P. vivax* : early schizonts in thick blood film stained for one second. The cytoplasm of the larger forms of *P. vivax* stains very intensely with brilliant cresyl blue. The field also contains one polymorph and two monocytes. Leucocytes are fairly well stained by this method ; their form tends to be better preserved than in films stained by standard Giemsa methods.

FIG. 3.—*P. falciparum* : gametocytes in thick blood film stained for one second. The cytoplasm of gametocytes is vividly defined even with this brief period of staining as penetration of the stain is almost immediate.

FIG. 4.—*P. malariae* : early schizonts in thick blood film stained for one second. The compact circular form usual in lysed and stained thick blood films is retained. The cytoplasm is well stained but the chromatin is poorly differentiated. The field also contains one leucocyte.

1

2

3

4

O   15 μ
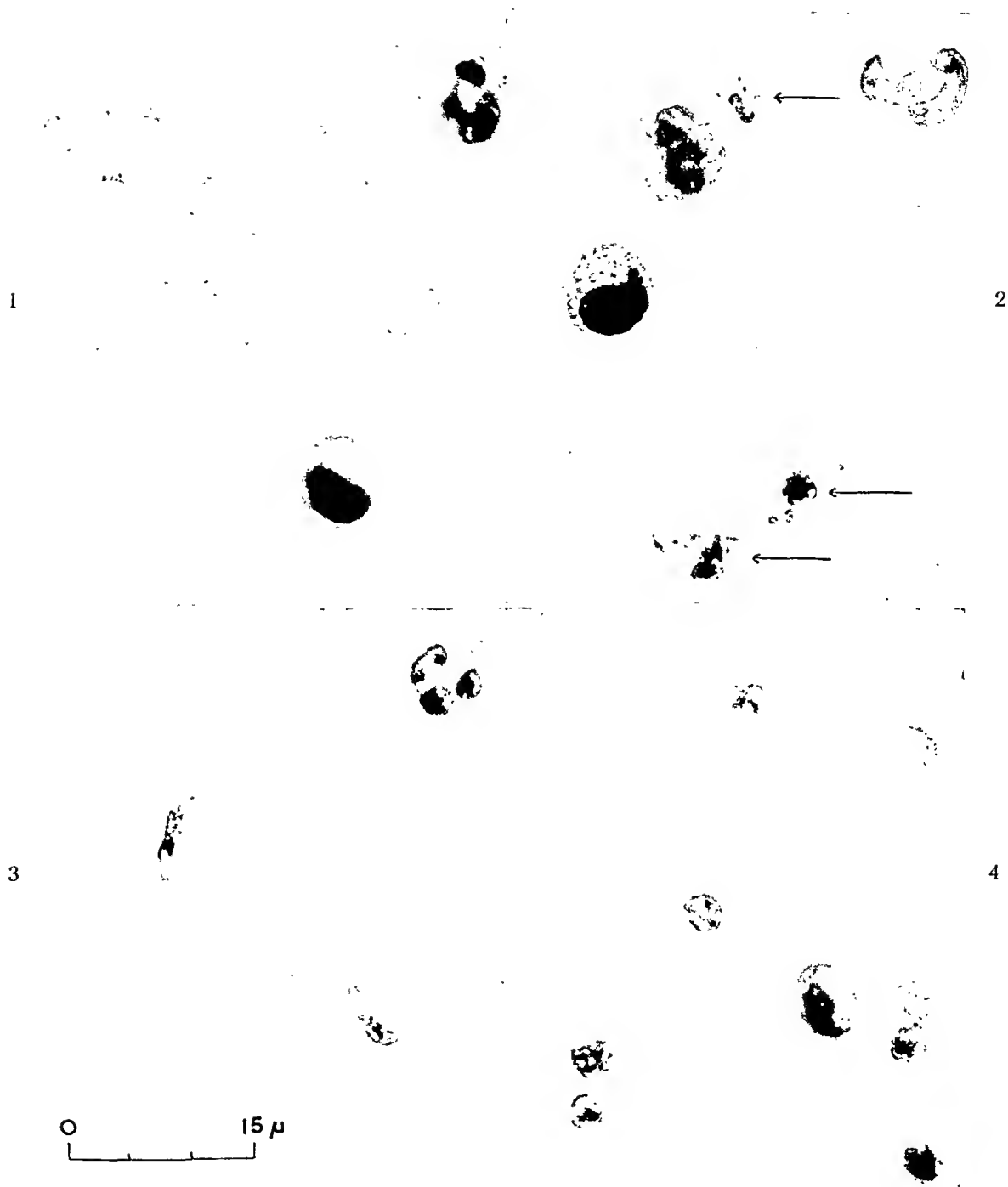
PHOTOMICROGRAPHS OF MALARIAL PARASITES IN THICK BLOOD FILMS STAINED FOR ONE
SECOND BY THE METHOD DESCRIBED IN THE TEXT.
(Objective 1/12 inch oil immersion, ocular × 15, Wratten B filter.)

Trophozoites.—Young trophozoites may preserve the ring forms usual in fixed thin films but are often collapsed. The cytoplasm is fairly deeply stained except with very young forms of *P. falciparum* which may be so faint that they are seen only with critical microscopy. The cytoplasm of *P. vivax* trophozoites is apt to appear broken and identification may be difficult until one is familiar with their appearance. The chromatin dot appears smaller than in Giemsa-stained films, is more faintly stained and undifferentiated in colour.

Schizonts.—Schizonts are deeply stained. Schizogony is less evident than in Giemsa-stained films but there is better preservation of outline. Generally speaking the larger the parasite the more intense the stain and schizonts are usually well contrasted against the creamy-yellow background.

Gametocytes.—The differentiation of gametocytes from the larger trophozoites or schizonts is sometimes difficult except where, as with *P. falciparum*, they have a distinctive shape. The chromatin is not well defined but the cytoplasm is deeply stained and the pigment is often seen well enough to afford assistance in identification.

The differentiation of species is little more difficult than in Giemsa-stained films; the indications which aid diagnosis are the same—the size of the parasites, the numbers present, the uniformity or diversity of phase, the compactness or dispersion of the cytoplasm, the amount, appearance and arrangement of the pigment, the number of merozoites in the segmenting forms, etc.

ADVANTAGES AND DRAWBACKS.

The one advantage of this rapid staining method over conventional methods is the extremely rapid diagnosis which its use permits. The smears are stained for 1 second only. Provided there are facilities for rapidly drying the blood, stained dried smears with all the advantages of a sixteen-fold concentration of parasites are available for examination in little over a minute after the blood is drawn.* The method has been adopted as a routine for immediate diagnosis in this laboratory and from tests in several hundreds of cases of acute malaria it has become clear that, for those who are familiar with the thick-smear morphology of malarial parasites, its use is consistent with fair accuracy.

There are two drawbacks: the minuteness and pallor of very young " ring " forms of *P. falciparum* and the fact that the stain is liable to emphasize the nuclear and reticular débris of immature red cells much more than is usual in Giemsa-stained smears. Young *P. vivax* trophozoites are sometimes found in immature cells with cytoplasm and reticulum so intermingled that the identification of the parasites is difficult, but a search for older forms will usually settle the diagnosis. Reticulocytes and the larger trophozoites of

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* Crescents are stained by this rapid technique with remarkable clarity of outline when the blood smears are rapidly made on a hot slide. Immediate heat fixation is, however, unsuitable for ring forms.

P. vivax have a superficial resemblance. Nuclear débris from immature red cells may be confused with parasite chromatin. Granted good microscopy, however, and a knowledge of the appearance of reticulocytes and of the nuclear residues of young red cells in thick smears of anaemic blood, these difficulties should seldom leave the diagnosis in doubt.

SUMMARY.

A method is described by which malarial parasites may be stained in thick blood smears in one second. Tests of this method in several hundreds of cases of acute malaria have shown that the great speed in malarial diagnosis which its use makes possible is consistent with fair accuracy.

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ATAXIC PARAPLEGIA OCCURRING AMONGST CHINESE IN MALAYA.

BY

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The main symptoms of the condition to be described are difficulty in walking and numbness of the legs. The difficulty in walking is largely due to ataxia and the above title has therefore been used for the syndrome although, as will be shown, it is not quite comprehensive as the disability is not entirely confined to the legs.

The study of this condition is a continuation of work that was carried out in collaboration with Dr. J. V. LANDOR some years ago (LANDOR and PALLISTER, 1935). We described, under the title of " Avitaminosis B2," the occurrence in institutions in Malaya of a disease that was characterized in the early stages by superficial glossitis, angular stomatitis and an eczematous condition of the scrotum. In the later stages of this disease nervous symptoms developed. The patients complained of pains, numbness and weakness of the legs and, in many cases, dimness of vision. The signs at this stage were a stocking type of anaesthesia, brisk tendon reflexes and some ataxia. It was noted in some of these cases, particularly among those of long standing, that the tendon reflexes were lost. As this earlier paper will be referred to frequently I will use the term " prison disease " to indicate the condition described there.

In the present paper a number of patients whose symptoms are similar to the last group mentioned are to be described ; that is to say they had sensory changes, absent knee-jerks and ataxia. Thus they are not quite the same as the majority of the patients seen with the prison disease but would seem to represent a definite condition that may arise in a certain section of the population. The patients were found among the general public and no institutional diet or mode of life can be blamed for their illness. They are apt to be mistaken for cases of beriberi, a diagnosis I believe to be incorrect, and it is for this reason and also to define what I consider to be one of the dietetic conditions of Malaya that this communication is submitted. Beriberi and pellagra are being recognized as complicating alcoholism and other conditions in Europe and America and it seems important that the nervous manifestations of deficiency diseases as seen in fairly large numbers in tropical countries should be described.

H. H. SCOTT (1918) recorded, under the title of " Central Neuritis," an

outbreak of disease amongst workers on sugar estates in Jamaica, whose nervous symptoms showed a close resemblance to those described in this paper. Amongst other symptoms the patients developed parasthesias, inco-ordination and an unsteady gait. The knee-jerks were absent. STANNUS (1936) has discussed this and many similar conditions in a more recent review.

DESCRIPTION OF CASES.

The following is a description of a typical case. The patient is a middle-aged Chinese and he complains of difficulty in walking and numbness of the legs of some months duration. He may, in addition, complain of some trouble with his hands especially if his work requires skilled movements. On examination he is found to be usually of ordinary nutrition without any distinct wasting of the limbs. The gait is rather unsteady and the feet placed a little apart for greater security. Though he may be able to get along fairly well at his own pace he is quite unable to hurry. The ataxia is not the same as a typical case of tabes dorsalis. The legs are not shot out in such an inco-ordinate manner and the knees are not hyper-extended, but the patient will, like the tabetic, watch carefully the ground in front of his feet. There is no foot-drop and the calves are not tender to pressure. In Rhomberg's position the patient is unsteady. The tendon reflexes are absent in the legs and Babinski's sign is flexor. Sensation is diminished or absent up to the ankles or knees and the position sense in the great toes is lost. In the arms the tendon reflexes may be present or absent and some sensory loss may be found about the hands.

A series of fifteen cases has been studied. These have been observed over some years in different hospitals. The fully developed condition cannot therefore be very common though minor degrees or less well-defined cases are not infrequently seen. All the patients observed were males. This may be partly explained by the fact that there is a preponderance of males in the country and it is also possible that the women are more usually able to get a variety of food. The patients were all Chinese. Somewhat similar complaints in other races in Malaya will be discussed later. The average age was 45 years, the youngest being 29 and the oldest 59. The occupations were varied, but all except one were labourers and belonged to the poorer classes. Enquiries were made into the diets but these seemed much the same as those of other people of the same class. Polished rice formed the basis of the diet and a small amount of vegetables, fish, pork and sometimes beans were taken in addition. Vegetables seemed low and the amount of animal protein was probably not very high but all partook of some. Questions regarding previous health were usually barren of results but two said they had suffered from weakness of the legs about 25 years previously when young men and one 4 years previously. Presumably these illnesses were beriberi. Five gave some history of oedema or dyspnoea but in no case was this severe. In two the history was vague and a third was very anaemic.

The duration of symptoms is inevitably rather uncertain in a chronic disease of this type occurring amongst a labouring class. Six said they had been troubled for 2 or 3 months and others gave various times up to $3\frac{1}{2}$ years. The average was just under 1 year. In two-thirds of the cases the complaints involved the upper extremities as well as the lower but the legs were the major trouble. On the sensory side the main complaint was numbness of the feet and legs but some spoke of tingling, cold feelings and vague pains. Weakness of the legs and difficulty in walking was what usually made them seek admission to hospital. One or two said that their walking was unsteady and one man expressed his symptoms more fully by saying that he had difficulty in stepping across a drain and that he could not always tell what he was walking on though he could distinguish sharp stones. In view of the complaint of poor vision among the men suffering from the prison disease special enquiries were directed to eyesight. Six made the complaint that their vision was poor. In one, at least, this was thought to be due to a local condition of the eyes and it may be that age contributed to some of the others but nevertheless some diminution of visual acuity appears to be sometimes present in association with the other symptoms. In two of these patients a slight pallor of the optic disc was present but in none was there blindness of such a degree to prevent them from getting about.

A history of diarrhoea was also sought, but in only four cases was this present. In one of these no free hydrochloric acid was present in the stomach even on stimulation with histamine. Of the remaining three two only were tested for acid and in them it was found present. None of the diarrhoeas was either serious or persistent but in the one without free acid the diarrhoea had occurred about the time given for the onset of the trouble with the legs and so possibly may have had some aetiological significance. It may be said, however, that diarrhoea is not an essential part of the syndrome. Cutaneous manifestations of pellagra were looked for. None of the patients showed the typical dermatitis of the wrists, ankles or neck but nine patients had a little angular stomatitis. Among these, slight changes in the tongue were noted in five. These changes consisted in a redness and swelling of the papillae along the margins and tip of the tongue. They were not severe enough to be the cause of complaint. Two patients and a possible third had eczema of the scrotum. No other special skin abnormalities were noted.

Of the general physical examination there was little abnormal apart from the nervous system. The heart and lungs were healthy though in one or two cases early arterio-sclerosis was present. Blood pressures have been recorded in nine cases and, of these, seven were within normal limits and two were below 100 mm. mercury (systolic). One of these suffered from diarrhoea. There was no evidence of malaria or of intestinal parasites except for one with round worms and there were no abnormalities in the urine.

The examination of the nervous system revealed definite changes. The cranial nerves were normal except for the occasional diminution in visual acuity

already referred to. No patient had Argyll-Robertson's pupils. In the arms muscular power was satisfactory and there was no obvious wasting except in one man whose hands showed a little wasting. The tendon reflexes were present in six and absent in nine. In one of the six the reflexes were slightly exaggerated and in another the biceps jerks were definitely brisk but the triceps jerks could not be obtained. In this case there was a slight difference between the two sides but in the others both sides were equal. Subjective signs are difficult to elicit in the class of patients here described ; in particular, the observations on vibration sense are apt to be faulty. There was no gross ataxia in the arms but one or two were a little unsteady in the finger-nose test. In eight there did not appear to be any loss of sensation to cotton-wool, pin or tuning fork. In the others there was some loss to cotton wool over the hands and wrists. This never extended far up the forearms. Diminution in pain sense was roughly the same as light touch. Vibration sense appeared to be absent in three or four. In the trunk, sensation was found to be normal except for loss of vibration sense over the lumbar vertebrae in five patients. In one of these the loss extended up into the dorsal region. The abdominal reflexes were present in the majority of the patients. There was no sphincter trouble. On examining the legs the muscular power was found to be reasonably good and there was no wasting. Muscles were sometimes flabby but there was never that degree of hypotonia that characterizes tabes dorsalis and in some cases there was a little increase in tone. The knee jerks and ankle jerks were absent in eleven of the fifteen patients. In one of the others the ankle jerk was present on one side, but the other ankle jerk and the knee jerks were absent. Of the remaining three the reflexes were rather exaggerated in two. No patient in the series had an extensor Babinski sign. Sensation to cotton wool was lost in twelve of the patients and in two others there was possibly some loss. The extent of the loss varied from the feet only in five up to the mid-thigh in three. Pain sense appeared to be normal in six but was blunted in the others. The sensory changes were approximately the same on the two sides and were of the " stocking " type. A fairly definite loss of vibration sense was noted in eleven patients and doubtful in three. The loss appeared more extensive than that of light touch and extended up to the lower vertebrae, as already mentioned, in some of the patients. The position sense in the great toes was examined in twelve patients and found absent in seven and doubtful in three. The gait has already been described in a typical case. All of the patients could walk but most of them liked to have the support of a stick. Rhomberg's sign was positive in thirteen and doubtful in one. The remaining patient differs from the others in that his gait was normal and he was steady in Rhomberg's position ; he must, therefore, be mentioned further. His complaints were poor eyesight and weakness and numbness of the legs. There was loss of light touch and pain to the middle of the calves and loss of vibration sense over the tibiae. The tendon reflexes were rather increased and he was one of those already mentioned as having

a possible mild optic atrophy. While unsteadiness of gait and a positive Rhomberg's sign have been taken as two of the main characteristics of the series this patient seems worthy of inclusion as being almost certainly of the same group especially in view of my previous observations on the prison disease. He provides what may be considered a link between this series and other similar conditions. Mentally, the patients appeared normal.

The Wassermann or Kahn test (often both) was carried out on the blood of thirteen of these patients. The result was negative in nine, doubtful in one and positive in three. The cerebrospinal fluid was examined in twelve and in only one was any abnormality found. Here there were 30 lymphocytes per c.mm. but the protein was within normal limits and the globulin test was negative. The possibility of an error in this case cannot be altogether eliminated. In ten patients the red count and haemoglobin estimation was carried out. One patient was anaemic with 2,780,000 red cells per c.mm. and 45 per cent. haemoglobin. One other had a high colour index, namely 1·2. His count was 3,100,000 and haemoglobin 75 per cent. The fractional test meal in this case showed the presence of free hydrochloric acid but at a low level. Ten patients had fractional test meals and eight had free acid. Of the other two only one was tested after histamine.

TREATMENT.

Treatment was given with the view that the disease was due to a dietetic deficiency. In addition to a satisfactory diet, marmite and fresh liver were usually given; and over a period of some months there was usually a little improvement in walking. The change was never enough to be easily measured. One patient received over a period of 24 days 57 c.c. of 5 per cent. nicotinic acid amide (Bayer) by injection, and later received 28 mg. of vitamin B_1 also by injection over a period of 4 weeks. Neither made any striking change, but the dosage is admittedly small. Liver extract and vitamin B_1 have been used for some other patients but never in massive doses and the benefit has been little. There is a wide and genuine use for these expensive drugs in the hospitals in Malaya and I have not felt justified in giving large doses over long periods to these patients with problematical benefit. I can make no deductions from the methods of treatment used except that the condition is not easily relieved.

DISCUSSION.

The age of the patients suggests a chronic infection, poisoning or food deficiency or alternatively a failure in middle age to utilise the ingredients of the diet as in Addisonian anaemia or as in the theory put forward by STANNUS (1937) in explanation of pellagra. I have not been able to find any evidence of infection. The resemblance to tabes dorsalis is quite superficial. The distribution of sensory loss is different, there is not the same hypotonia and there are no pupillary changes. The blood tests and the results of the examinations of the cerebrospinal fluids are against infection. Of some possible poisoning

The typical case in each group differs a little but there are intermediate cases so it seems that each group merges into the other two. There can be little doubt that the three conditions are closely related ; and if diet is at fault, then there is some similar deficiency which is modified slightly by other factors.

Is ataxic paraplegia a form of pellagra ? HARRIS (1919) uses the term " pellagra *sine* pellagra " and says he "thinks there is not a shadow of doubt of the very frequent occurrence of pellagra without skin lesions," a view supported by STANNUS (1936). In a fairly recent paper by BIGGAM and GHALIOUNGUI (1933) the main features of pellagra as it affects the nervous system are described. They say that on the sensory side paraesthesias, tingling and numbness, especially in the lower limbs, are the commonest manifestations. A burning sensation in these parts is sometimes noted. Objective sensory loss and Rhombergism are rare. On the motor side stiffness and weakness, associated with increased reflexes is the change most frequently found. Ataxia is not uncommon. The manifestations are very varied and perhaps more resemble " burning feet " though there are clearly points of resemblance to ataxic paraplegia. In an earlier number of these TRANSACTIONS, SHELLEY (1930) in speaking of pellagra in Nyasaland says, " the reflexes are usually increased and brisk in the early stages and sluggish or absent in the later stages. In advanced cases the patient walks on a broad base but the gait is not definitely ataxic." He divides his cases into four clinical types and one he describes as the paralytic type. GREENFIELD and MACDONALD HOLMES (1939) discussing the pathological changes in pellagra say, " it would appear that the most common lesions in the spinal cord consist of symmetrical degeneration of the dorsal columns, especially that of Goll, and the spino-cerebellar and pyramidal tracts. In most cases the afferent tracts are more affected than the efferent. . . ." No opportunity has occurred of obtaining a postmortem examination in the present series. A piece of the terminal branch of the deep peroneal nerve from one patient was examined by the Weigert-Pal stain and showed considerable degeneration.

It would seem that in typical pellagra spasticity and brisk tendon reflexes are the nervous lesions most commonly found on clinical examination, but that ataxia and absent reflexes may occur. The pathological findings show the extent to which the afferent tracts are actually damaged. There can be little doubt that the symptoms of the patients here described are not incompatible with the diagnosis of pellagra. It will be remembered, too, that mouth and scrotum lesions were noted among some of the cases and these have been shown (LANDOR and PALLISTER, 1935) to be due to a vitamin B_2 deficiency. STANNUS (1936), in an exhaustive review, has discussed many obscure tropical diseases under the title of " Pellagra and Pellagra-like Conditions." He considers that the prison disease, " burning feet " and also the " central neuritis " of SCOTT (1918), already mentioned as resembling in some features ataxic paraplegia, are in reality pellagra. If we may assume for the present that pellagra is a disease or group of diseases whose manifestations vary somewhat in different localities

then it seems right to include ataxic paraplegia as one of the group. This is perhaps hardly satisfactory as a final diagnosis but the work that is being done on nicotinic acid and other constituents of the vitamin B complex may be expected to throw more light on these conditions.

SUMMARY.

1. A disease occurring among Chinese in Malaya is described. The main complaints are weakness and numbness of the legs; and the main signs absent tendon reflexes, sensory loss and ataxia.

2. The aetiology is discussed and the disease is thought to be a form of pellagra modified by other factors in the diet or circumstances of those affected.

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STUDIES IN LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN.

IV.—A PUNCTATE RASH IN TREATED CASES.

BY

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AND

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In a previous communication (KIRK and DREW, 1938) there was described a finely punctate eruption which appears in cases of kala-azar during the course of treatment with antimony. This eruption is of relatively common occurrence in the Sudan, being observed in about 20 per cent. of treated cases, and is of considerable interest, for as far as the authors are aware a similar rash in man has not been described in the other endemic centres of leishmaniasis. Owing to the difficulty of demonstrating parasites histologically in this rash it has been the custom in the past to regard it as due to antimony rather than to the leishmanial infection, but observations recently made have shown that this view is not entirely correct.

CLINICAL DESCRIPTION OF THE RASH.

The eruption becomes evident in cases of visceral kala-azar during treatment, usually after the first or during the second " course ", *i.e.* after fifteen to thirty injections of neostibosan, the most commonly used preparation. (Fig. 1.) In the experience of the writers the eruption is seen only in cases which are reacting favourably to the treatment, and may therefore be regarded as having a good prognostic significance. It appears first on the face, usually on the forehead or malar region, as a minutely punctate rash. Sometimes its distribution may be restricted to those regions, but most commonly the whole face and neck become involved. In the coarser forms of the eruption the punctae are larger, readily palpable, and could more accurately be described as papules. In some cases miliary sudamina accompany the rash, but in others it may be difficult to distinguish individual punctae and the rash has the appearance of xerodermia or a mild ichthyosis of the affected parts. Ulceration does not occur. Sometimes the rash extends downwards to the trunk, but when this occurs it is usually more prominent on the face than elsewhere, and tends to fade as it passes downwards over the trunk. Occasionally it can be observed on the legs. There are no subjective symptoms, such as itching, and often the rash passes unnoticed by the patient until his attention is directed to it by the medical attendant.

Contrary to the post-kala-azar dermal leishmaniasis of India this rash tends to disappear spontaneously within a few months after discharge from hospital.

CAUSE OF THE RASH.

As far as present observations go the rash develops only in cases which have had a certain amount of treatment, but this point demands further investigation. Hitherto the only specific drugs used in treatment have been trivalent or pentavalent antimony compounds. The typical antimony rashes described in the text books are urticarial or pustular, and bear little resemblance to the condition described above. Yet some of the writers' colleagues state that occasionally they have observed similar punctate eruptions in cases of schistosomiasis, outside the kala-azar areas, before and during treatment with antimony. For this reason it has hitherto been difficult to exclude the possibility that antimony *per se* might be the cause of these eruptions. Recently, however, similar eruptions have been observed in five out of twenty cases of kala-azar successfully treated with 4 : 4'-diamidino stilbene, an aromatic compound (cf. LOURIE and YORKE, 1939) which contains no antimony. (Figs. 2, 3 and 4.) Hence in these five cases at least it is evident that the eruption was not due to antimony, although clinically it was indistinguishable from the eruptions seen in cases treated by neostibosan or tartar emetic.

A certain amount of evidence seems to support the conclusion that the primary cause of the rash is the leishmanial infection. Parasites are not readily found in the lesions, but they have been demonstrated in seven instances, or 12 per cent. of the cases investigated. The only method employed has been the microscopical examination of stained smears made from skin scrapings. It is possible that cultural methods might reveal parasites in a larger proportion of cases, but cultural methods are unsatisfactory owing to the difficulty of excluding blood, or cells from the subcutaneous tissues.

The clinical resemblance between this punctate eruption and the macular depigmented rash of post-kala-azar dermal leishmaniasis depicted by NAPIER and DAS GUPTA (1930) is also significant. It may be suggested further that the difference between the present punctate rash and the more typical nodular eruption depicted in an earlier paper (KIRK and DREW, 1938) is one of degree only. Support is given to this view by four cases observed by the writers. In three of these (one of which is described in the earlier paper) the more typical nodular eruption appeared during the course of treatment instead of the finer punctate rash. In the fourth there appeared simultaneously a typical nodular eruption on the face with a minutely punctate rash over the remainder of the body surface.

NATURE OF THE RASH.

Although the presence of leishmania in this rash has now been demonstrated in a number of instances, caution must be exercised in the interpretation of positive findings. The appearance of cutaneous lesions during the course of treatment is by no means completely explained by the discovery of parasites in a small proportion of the lesions, since it has been shown (KIRK and SATI, 1940)

FIG. 1.

FIG. 2.
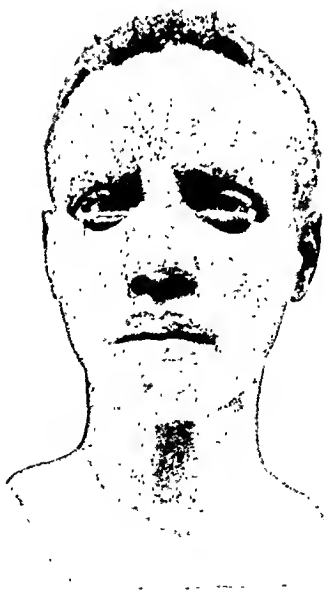
FIG. 3.

FIG. 4.

EXAMPLES OF PUNCTATE RASH.

The eruption shown in Fig. 1 developed during treatment with neostibosan, those in Figs. 2, 3 and 4 during treatment with 4 : 4'-diamidino stilbene.

that the parasites are frequently present in the skin during the course of the visceral infection, and before treatment has been started.

According to AUGIER and FAURE-BRAC (1935) the cutaneous lesions which occur frequently in canine kala-azar are temporarily increased during treatment with various antimony compounds, usually after ten to sixteen injections have been given. It is stated that the parasites are more readily found in the cutaneous lesions during this " poussée de dermatite," which is sometimes accompanied by an access of fever. These writers regard this exacerbation under the influence of chemotherapy as comparable to the Herxheimer reaction in syphilis, and in their search for an analogous phenomenon in the pathology of human kala-azar cite the cutaneous pigmentation described by D'OELSNITZ (1933) which, it is stated, is seen only in treated cases. The present writers have noted similar pigmentation in cases where the disease progressed steadily to a fatal termination as well as in untreated cases, and suspect that these pigmentary changes are due to a different cause. But it may be suggested that the appearance of punctate or frankly nodular skin eruptions in human cases after fifteen to thirty injections of antimonials bears some analogy to the exacerbation of the skin lesions in canine kala-azar after a similar course of treatment. In the human cases no constant association has been established between the appearance of the punctate eruptions and exacerbations of fever. In this connection, however, mention may be made of two cases observed by the present writers, in which nodular eruptions appeared on the face during treatment. Parasites had previously been demonstrated in very inconspicuous lesions in the same situation before treatment was started. A description of the clinical features and course of the nodular eruptions lies outside the scope of the present paper, but it may be noted that one of the cases was treated with neostibosan and the other with 4 : 4'-diamidino stilbene. In the latter the development of the eruption was accompanied by a relapse of the fever over a period of eight days with epistaxis, and a temporary re-enlargement of the spleen. If it is difficult to regard the eruption simply as a drug rash, it is equally difficult to attribute it solely to the presence of the parasites in the skin since they were present in the latter situation before the eruption appeared. The appearance of exuberant cutaneous manifestations in association with epistaxis and a temporary exacerbation of the visceral condition seems to indicate some allergic or Herxheimer reaction, the mechanism of which is obscure.

DISCUSSION.

Attempts to demonstrate infection of the skin and lymphatic glands during the visceral stage of kala-azar in India have almost invariably given negative results (NAPIER, 1935), but post-kala-azar dermal leishmaniasis occurs not uncommonly 1 to 2 years after the visceral disease has subsided, and may assume a variety of clinical forms. In some instances (NAPIER, 1935) infection of the skin has been demonstrated in the absence of any clinical manifestations whatsoever, by feeding sandflies on clinically cured cases. NAPIER and KRISHNAN (1933) have suggested that dermal infection is a natural sequel to visceral infection,

but that only in a small percentage of persons does the dermal infection reach the clinical stage. They regard the dermal lesions as evidence of an imperfect immunity response, a compromise between susceptibility and established tolerance. The latter is visualized as a state of affairs in which the parasites are present in the skin, producing no clinical lesions, but still capable of infecting sandflies and thus maintaining the cycle of infection.

In the Sudan the evolution of kala-azar in the human subject apparently follows a different course. The latent period of 1 to 2 years between the visceral and dermal manifestations in treated cases is unusual—more commonly a relapse of the original visceral condition occurs within this period. Infections of the skin and superficial lymph glands frequently accompany the visceral disease in the Sudan. Sometimes cutaneous infection is found when an untreated case of kala-azar first comes under observation. In other instances dermal lesions appear during the course of treatment, and a certain number of these closely resemble the Indian conditions.

In the present state of knowledge of the manner in which drugs effect clinical cure in kala-azar, speculation about the mechanism by which new dermal lesions are produced during the course of treatment is unsatisfactory. If it be the expression of an imperfect immunity response in the skin it is difficult to understand why this should become manifest at a time when the visceral disease begins to abate. As far as the writers are aware only one other phenomenon has been described which is comparable to the appearance of the punctate and nodular eruptions during treatment in the Sudan cases. This is the exacerbation of the skin lesions during treatment in canine kala-azar described by AUGIER and FAURE-BRAC (1935) who regard the phenomenon as analogous to the Herxheimer reaction.

SUMMARY

1. A description is given of a punctate cutaneous eruption commonly found in cases of kala-azar in the Sudan, and appearing during treatment.

2. Proof is given that the rash may occur in the absence of antimony, and hence it cannot be a specific toxic effect of this drug. Reference is made to an apparently allied phenomenon in canine kala-azar during treatment.

3. The relation between this rash and the post-kala-azar dermal leishmaniasis of India is discussed, and it is concluded that there are differences in the evolution of Indian and Sudan kala-azar in man.

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THREE CASES OF TRYPANOSOMIASIS RELAPSING DURING TREATMENT WITH BAYER 205 (GERMANIN).

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This paper describes three cases of human trypanosomiasis which relapsed during or shortly after a course of treatment with Bayer 205, and in which chemical estimations were made of the concentration of the compound in the blood. The cases were observed at Kahama, in the Western Province of Tanganyika Territory, East Africa, in which region sleeping sickness of the *Trypanosoma rhodesiense* type is endemic.

CASE I.

Masala, daughter of Manyenye ; aged 80 ; a new case ; village Iuzungunye. First seen Nov. 17th, 1938.

History.—Had been ill 1 month. Lacked strength and was unable to work. Complained of swelling of hands and feet ; pains in chest, arms and legs ; cough ; loss of appetite.

Nov. 17th.—*Examination.*—Nourishment fair ; face somewhat swollen ; intelligence normal ; ambulant. Weight 45 kg. Lymph glands palpable in axillae and groins (about 1 to 2 cm. across). Heart, slight systolic murmur. Pulse rate, 104. Abdomen, rather prominent and lax ; spleen enlarged 8 cm. ; liver enlarged 1 cm. Feet showed some oedema. Blood, haemoglobin 85 per cent. (Tallqvist) ; scanty trypanosomes present. One ml. of citrated blood was injected intraperitoneally into each of two rats ; both became infected in less than 8 days. Urine contained albumin.

Nov. 17th (11.0 a.m.).—Given Bayer 205, 1 gramme, intravenously.

Nov. 18th.—Cerebrospinal fluid, trypanosomes absent ; cells, 84 per mm.^{3} ; protein, 20 mg. per 100 ml. Concentration of Bayer 205 in C.S.F. (10.30 a.m.), nil. Concentration of Bayer 205 in blood (10.30 a.m.), 8·5 mg.† per 100 ml.

Nov. 22nd. and 26th.—Bayer 205, 1 gramme.

Nov. 30th.—Tryparsamide, 2 grammes i.v. (in connection with another series of observations).

Dec. 1st.—C.S.F. ; cells, 18 per mm.^{3} ; protein 20 mg. per 100 ml.

Dec. 8th, 12th and 18th.—Bayer 205, 1 gramme.

Dec. 27th.—Tryparsamide, 2 grammes.

Pyrexia was present from Dec. 16th to 24th.

Dec. 18th.—Malaria parasites were present in the blood, but examinations on Dec. 23rd and 24th showed no parasites. Examination of urine on Dec. 24th showed the presence of protein.

Dec. 27th.—Temperature 102·2° F.

† *Note.*—In all figures for the concentration of Bayer 205 in the plasma, a blank value, corresponding to 0·84 mg. per 100 ml., has been subtracted from the actual reading obtaining ; see previous paper (HAWKING, 1940). With figures for the C.S.F., no blank value is required.

Dec. 28th.—Temperature 101·0° F. Pulse 132. Headache and pain in chest behind manubrium sterni. Cough. Conjunctivae yellowish.

Blood contained numerous trypanosomes (about five in a microscopic field with the oil-immersion lens, thick film).—The trypanosomes were mostly forms of moderate length. 0·8 ml. of blood was injected intraperitoneally into each of three rats ; none became infected.

Concentration of Bayer 205 in blood, 4·5 mg. per 100 ml.

Haemoglobin 60 per cent. White blood corpuscles 15,400 per mm.³ ; Differential count : Polymorphs 66 per cent., Lymphocytes 30 per cent., Monocytes 4 per cent., Eosinophils 0 per cent. Urine darkly pigmented, and contained protein (48 mg. per 100 ml.).

Dec. 29th.—Temperature 98·0° F. Pulse 96. Patient felt all right except for moderate pain in the chest. Blood, no parasites. Weight 40 kg.

Dec. 30th.—Temperature 103·4° F. Blood, no parasites.

Dec. 31st.—Temperature 98·8° F. Pulse 100. Patient felt unwell ; slight pain in chest and cough ; pain in stomach. Abdomen appeared normal. Conjunctivae definitely jaundiced. Blood, no parasites.

Given Bayer 205, 1 gramme, 10 a.m.

Jan. 2nd.—Temperature 98·0° F. Pulse 96. Patient felt normal. Conjunctivae slightly less jaundiced, but serum was obviously stained with bile.

Concentration of Bayer 205 in blood (10 *a.m.*), 4·2 mg. per 100 ml.

After this date, the writer left the district and observation was continued by the Sub-Assistant Surgeon, Mr. G. V. GODBOLE.

On **Jan. 7th,** patient received 1 gramme, Bayer 205. From **Dec. 31st** until **Jan. 9th,** the temperature remained normal, and on **Jan. 9th** the patient returned to her own village to continue treatment.

CASE 2.

Maduhu, son of Kajala ; aged 40 ; new case. First seen Dec. 10th, 1938.

History.—Began 6 weeks ago with diarrhoea and vomiting ; later headache which has continued. Six days ago, pain in eyes and impaired vision.

Examination (**Dec. 10th**).—Nourishment fair, face slightly swollen ; intelligence fair ; ambulant. Weight (Dec. 28th), 50 kg. Lymph glands, numerous, palpable in axillae ; a few palpable in inguinal and femoral regions. Pulse 138. Abdomen, liver and spleen just palpable. Blood, haemoglobin (Tallqvist) 80 per cent ; scanty trypanosomes present ; inoculated into two rats which became infected within 7 days. Urine contained a trace of protein.

C.S.F. (Dec. 12th), trypanosomes absent ; cells, 50 per mm.³ ; protein, 42 mg. per 100 ml.

Dec. 10th, 14th, and **17th.**—Bayer 205, 1 gramme, intravenously.

Dec. 21st.—Pyrexia.

Dec. 22nd.—Pyrexia.

Blood contained trypanosomes in moderate numbers.

Concentration of Bayer 205 in blood : 1·0 mg. per 100 ml. (Estimation made in duplicate). One ml. of blood injected into each of three rats which became infected in less than 9 days.

Dec. 23rd.—Temperature 101·8° F. Pulse 136. Patient had slight headache and pain in chest. The pain was pronounced over the sternum at the level of the third rib, and was less marked on both sides ; there was no suggestion of a special praecordial localization.

Blood contained trypanosomes, about two to four per microscope field, with an oil-immersion lens and a thick film. The trypanosomes seemed morphologically normal ; no dividing forms were seen. Urine contained a slight trace of protein.

Dec. 25th.—Temperature 98·4° F. Blood, no parasites.

Dec. 26th.—Temperature 98·0° F. Pulse 80. Patient felt normal. Blood, leucocytes, 9·900 per mm.³.

Differential count : Polymorphs 52 per cent., Lymphocytes 34 per cent., Monocytes 10 per cent., Eosinophils 4 per cent.

Concentration of Bayer 205 in blood (10.45 a.m.) : 1·0 mg. per 100 ml. (10.45 a.m.) Bayer 205, 1 gramme.

Dec. 28th (11.0 a.m.).—*Concentration of Bayer* 205 *in blood* : 1·5 mg. per 100 ml. (11.0 a.m.) : Bayer 205, 1 gramme.

Dec. 29th (9.30 a.m.).—*Concentration of Bayer* 205 *in blood* : 1·5 mg. per 100 ml.

Dec. 30th (11.0 a.m.).—Bayer 205, 1 gramme.

Jan. 2nd (9.30 a.m.).—*Concentration of Bayer* 205 *in blood* : 1·0 mg. per 100 ml.

After this date, observations were continued by Mr. GODBOLE. Tryparsamide, 2 grammes, given on **Jan. 9th, 16th and 23rd.** On **Jan. 24th,** general condition was good ; weight, 50 kg. ; urine contained no protein ; there had been no relapse and no return of pyrexia. Treatment with tryparsamide was continued.

CASE 3.

Mabula, son of Kisa ; aged 30 ; new case. First seen Dec. 6th, 1938.

History.—Three months ago began to have pain in right upper arm ; later the legs swelled and became heavy. Discomfort in epigastrium and inability to work on account of weakness.

Examination (**Dec. 6th**).—Nourishment poor ; face swollen ; apathetic ; could walk slowly ; weight, 50 kg. Lymph glands palpable in axillary, epitrochlear, and inguinal regions (small) and in femoral regions (about 3 cm.). Heart : systolic murmur ; pulse 100. Abdomen : liver enlarged about 1 cm. and spleen 2 cm. Legs, wasting ; oedema of ankles. Blood : haemoglobin (Tallqvist) 80 per cent. ; many trypanosomes present. One ml. injected into each of two rats, which became infected in less than 7 days. Urine contained no trace of protein.

C.S.F. (Dec. 7th), trypanosomes, 8 per mm.^{3} ; cells, 30 per mm.^{3} ; protein, 27 mg per 100 ml.

Dec. 6th and 9th.—Bayer 205, 1 gramme injected intravenously.

Dec. 16th and 23rd.—Tryparsamide, 2 grammes.

Patient improved gradually until Dec. 28th.

Dec. 28th.—Temperature 101° F. ; pulse 100. Severe headache.

Blood contained numerous trypanosomes (about two per microscope field, using an oil-immersion lens on a thick film) ; many small thin forms were present ; 0·8 ml. of blood inoculated into each of three rats which became infected after 16 to 20 days.

Concentration of Bayer 205 *in plasma* : 0·15 mg. per 100 ml. (*i.e.* within the range of values found with plasma from untreated patients).

Haemoglobin 90 per cent. ; white blood corpuscles, 11,200 per mm.^{3} Differential count : Polymorphs 54 per cent., Lymphocytes 38 per cent., Monocytes 6 per cent., Eosinophils 2 per cent.

Urine contained protein, 62 mg. per 100 ml.

Dec. 29th.—Temperature 99·0° F. Pulse 68. Patient felt normal. Weight 49 kg.

Blood contained very scanty trypanosomes.

Dec. 30th.—Temperature 99·0° F. Blood, no parasites.

Dec. 31st.—Temperature 100·0° F. Pulse 80. Blood contained malaria parasites but no trypanosomes. (10 a.m.) Bayer 205, 1 gramme.

Jan. 2nd.—Temperature 98·0° F. Pulse 60. Patient felt normal.

Concentration of Bayer 205 *in plasma* : (9.30 a.m.) 0.

After this date, observations were continued by Mr. GODBOLE. Bayer 205 was given on Jan. 2nd, 7th, 14th and 21st.

Jan. 24th.—General condition was good ; weight 44 kg. ; urine contained no protein ; there had been no relapse, and no return of pyrexia. Treatment was continued with tryparsamide.

The main features of the three cases above may be summarized thus :—

All were new cases, with a duration between 1 to 3 months ; in all the cerebrospinal fluid was affected to a moderate extent. At the time they relapsed, the first had received six doses of Bayer 205, from 9 to 41 days previously ; the second had received three doses from 5 to 12 days previously ; and the third had received two doses, from 19 to 22 days previously. In each case, the

reappearance of trypanosomes in the blood was signalized by a clinical disturbance : pyrexia, headache, and pains in the chest. Although no further treatment was given at the time, trypanosomes disappeared from the blood spontaneously after 1 to 3 days. In all cases, estimations of the concentration of Bayer 205 in the plasma showed amounts much smaller than would have been expected from experience with other patients (HAWKING, 1940), and when further doses of Bayer 205 were given, the drug did not accumulate in the blood at the usual rate. The first case, *Masalu*, differs in some respects from the other cases ; dosage previous to the relapse had been greater, and larger concentrations of the compound were found in the blood ; the blood containing trypanosomes taken at the time of the relapse failed to infect rats ; and the relapse was accompanied by jaundice indicating damage to the liver. The fact that the trypanosomes of these patients disappeared again so quickly without further treatment, gives the impression that in some way the defence mechanism (patient plus drug) had undergone a temporary paralysis so that parasites swarmed out into the peripheral blood unopposed ; then the defence mechanism regained its normal efficiency and the trypanosomes were quickly brought under control again. The presence of pain behind the sternum in two of these relapses is interesting in view of the myocarditis which has been described in the trypanosomiasis of animals.

The possible explanations for these unusual occurrences are as follows :—

1. *Abnormality of the trypanosome-strains.*

(a) *Morphology.*—This was studied carefully in blood films taken from the patients' blood at the time of relapse, and the results are recorded above in the course of the clinical descriptions. No definite abnormalities were observed, although the absence of dividing forms (noted in Case 2), is unusual. VON JANCSÓ and VON JANCSÓ (1934), studying the effects of Bayer 205 in animals after blockade of the reticulo-endothelial system, described multinuclear forms, caused by division of the nucleus without division of the cytoplasm. No such forms were observed in these cases.

(b) *Virulence.*—In two of these three instances the trypanosomes, taken at the relapse, were able to infect rats. Blood taken from Case 1 at the relapse failed to infect three rats although it contained numerous living trypanosomes. This blood contained Bayer 205 in a concentration of 4·5 mg. per 100 ml. which may have been sufficient to prevent infection of a new animal (HAWKING, 1939), although in that case it is remarkable that the parasites were able to reappear in the patient's blood at all. The trypanosomes of Case 3, taken at the time of relapse, also seem to have had some difficulty in infecting the rats, since the incubation period was 16 to 21 days instead of the usual 7 days ; the concentration of Bayer 205 in the plasma at this time was too low to be estimated.

The length of life of rats inoculated from these patients, dating from the time of inoculation, was as follows :—

Case 1—15 and 16 days ; average 15·5.

Case 2—33 and 44 days ; average 38. After relapse, 23, 25 and 30 days ; average 26.

Case 3—16 and 23 days ; in second passage, 17, 14 and 21 days ; average 18. After relapse, 39, 39 and 52 days ; average 43. (These animals had a long incubation period of 16 to 21 days ; usually the incubation period was less than 7 days.

The average incubation periods of seven groups of two to three rats (each group being inoculated with blood from one of seven other new patients who all gave the expected response to Bayer 205) were : 14, 17, 29, 30, 34 and 81 days. Thus there is no suggestion that the virulence (for rats) of the strains in question was abnormally high or low for this district.

(c) *Resistance of trypanosomes to Bayer* 205.—The trypanosomes from the two cases in which rats were infected at the time of relapse were tested for their sensitivity to Bayer 205 *in vivo*. Rats were infected from those of the first passage and after about 4 days, when the blood usually contained one to twenty trypanosomes per microscope field (1/6 objective, 2 ocular) in a cover-slip preparation, they were injected intraperitoneally with suitable doses of Bayer 205 dissolved in about 1 ml. of water. The blood was examined for the next 4 days ; the minimum effective dose was taken as that dose which cleared the blood of trypanosomes in a majority of the animals on two successive days during this period (see also a subsequent paper*). The results are shown in the accompanying table, which also gives the results with trypanosomes from five other patients for comparison. Two of the latter were old cases who had relapsed in spite of repeated courses of treatment with Bayer 205 during many years, and in whom Bayer-resistant strains might have been expected. The table shows that the strains from *Maduhu* and *Mabula* (Cases 2 and 3) showed normal sensitivity to Bayer 205. Consequently the relapses which occurred in their cases cannot be assigned to undue Bayer-resistance on the part of their trypanosomes.

2. *Abnormality of the defence mechanism of the host.*

According to the work of von Jancsó and von Jancsó (1934), and others, it seems probable that the therapeutic action of Bayer 205 is produced by exerting an opsonin-like effect upon the trypanosomes which are then more readily engulfed by the phagocytes of the host. Consequently the possibility must be considered that the relapses were due to some defect in the phagocytes of these patients. Examinations of the leucocytes of the blood, by total and differential counts, as described above, revealed nothing abnormal. In Case 1, jaundice occurred which might conceivably be associated primarily or secondarily with some pathological condition of the Kupffer cells of the liver ; but it should be noted that the jaundice *followed* the reappearance of the trypanosomes instead of *preceding* it as would have been expected in this case. Altogether, it is con-

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cluded that although an abnormality of the defence-mechanisms cannot be excluded, no evidence could be found for its existence.

3. *Abnormality in the samples of compound used.*

During the second half of 1938, the batches of Bayer 205 (Germanin) in use at the Kahama Hospital were Nos. 680895, 781199 and 735422. These had

TABLE.

SHOWING THE MINIMUM EFFECTIVE DOSE (M.E.D.) OF BAYER 205 REQUIRED TO STERILIZE THE BLOOD OF RATS INFECTED WITH TRYPANOSOMES FROM VARIOUS PATIENTS.

Patient.	Dose. Mg. per 100 grammes.	Degree of infection. Parasites per field.*	Proportion of animals which became negative.				M.E.D. Mg. per 100 grammes.
			1st day.	2nd day.	3rd day.	4th day.	
Maduhu Kajala (after relapse)	0·05 0·1 0·2	2 to 10 1 to 5 1 to 20	1/3 1/5 4/4	0/3 5/5 4/4	0/3 3/5 4/4	0/3 0/5 2/4	0·1+
Mabula Kisa ... ... (after relapse) ...	0·05 0·1 0·2	1 to 10 1 to 5 5	0/5 2/4 1/1	1/5† 4/4 1/1	1/5 4/4 1/1	1/5 4/4 1/1	0·1
Nduthi Kafuru ... (new case)	0·05 0·1	1 to + 2 to +	0/5 0/4	2/5 4/4	1/5 3/4	0/5 1/4	.0·1
Bijirimana Ntambigamba (new case)	0·05 0·1 0·2	5 to 30 1 to 30 10 to +	0/2 1/4 3/4	0/2 1/4 4/4	0/2 0/4 1/4‡	0/2 0/4 1/4	0·2+
Maganga Bugamba ... (new case)	0·05 0·1	5 to 20 1 to +	0/3 1/4	0/3 4/4	0/3 3/4	0/3 3/4§	0·1
Maganda Mihambo ... (old case)	0·025 0·05 0·1 0·2 0·4	1 to 3 2 to 15 5 to 10 10 3 to 10	0/4 1/4 0/2 1/2 1/2	1/4 2/4 2/2 2/2 2/2	1/4 3/4 2/2 2/2 2/2	0/4 4/4 2/2 2/2 2/2	0·05
Georgi Luganda ... (old case)	0·025 0·05 0·1 0·2	5 to 10 ⅓ to 10 3 to + 5 to 10	0/2 1/5 0/3 0/2	0/2 0/5 3/3 2/2	1/2 1/5 3/3 2/2	1/2 2/5 3/3 2/2	0·1

* Microscope field (1/6 obj., 2 ocular). + indicates about 30 to 100 parasites per field.
† This was a different animal to the one shown on the 2 succeeding days.
‡ This animal became negative on the 2nd day.
§ One of these was a different animal to those of the previous day.

all been issued in 1935 as sealed glass ampoules each containing 1 gramme ; for most of the succeeding period they had presumably been stored at tropical temperatures. Specimen ampoules from two of these batches were returned to the Bayer Laboratories with the request that their therapeutic potency should be retested ; but no reply had been received before the outbreak of war. However, some evidence for the potency of these specimens used may be obtained in other ways. During this period of 7 months, twenty-one other new cases of sleeping sickness were treated with these preparations and the usual clinical responses were obtained. The drug used in treating the rats infected with trypanosomes from *Maganda*, *Maganga* and *Georgi Luganda* (see Table) seems to have been undiminished in therapeutic potency. Finally a number of chemical estimations made by the Wormall method (DANGERFIELD, GAUNT and WORMALL, 1938) during this period on solutions made from these batches, all yielded the expected result. Although none of these pieces of circumstantial evidence is conclusive and it is possible that the compound in the particular ampoules used for treating these patients may have deteriorated, yet it seems unlikely that such deterioration is the explanation of the relapses which occurred, especially since similar relapses have been observed by numerous other workers (see below).

4. *Abnormality in the accumulation of Bayer 205 in the blood of these patients.*

Estimations were made of the concentration of Bayer 205 in the blood of these patients on the day of relapse, and on various subsequent occasions, and the above results have been recorded in the course of the clinical histories. The estimations may be summarized as follows :—

	Concentration in blood mg. per 100 ml.	Days since injections.
Case 1	3·5	1·0
	4·5 (trypanosomes present)	9, 16, 23, 32, 36, 41
	4·2	2, 14, 21, 28, 37, 41, 46
Case 2	1·0 (trypanosomes present)	5, 8, 12
	1·0	9, 12, 16
	1·5	2, 11, 14, 18
	1·5	1, 3, 12, 16, 19
	1·0	3, 5, 7, 16, 20, 23
Case 3	0·15 (trypanosomes present)	19, 22
	0·0	2, 24, 27

These results should be compared with those obtained from other patients who responded normally to treatment, which have been recorded (HAWKING,

1940) in the earlier paper (Figs. I, III and IV), and the graph of the blood concentration in Case 2 of the present paper should be placed against Fig. II of the earlier paper which is drawn to the same scale. The first observation on Case 1 comes well within normal limits, *viz.*, 2·5 to 5·8, average 4·1 mg. per 100 ml. (five patients) ; the other two observations on this case, although within the range observed during the first 10 days after courses of four injections, *viz.*, 3·2 to 15·2 mg. (Fig. III of the earlier paper) are distinctly below the average of such results, *viz.*, 8 mg. per 100 ml. (thirteen patients). In particular, it is remarkable that the injection given on Dec. 31st had no appreciable effect in raising the blood concentration, as judged by the observation made 2 days later.
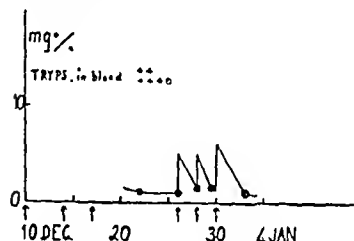
Graph showing the concentration of Bayer 205 in the blood of Case 2 (*Maduhu Kajala*).

The time of each injection (1 gramme) is indicated by the arrows. This figure should be compared with Fig. 2 of the earlier paper which is drawn to the same scale.

The figures obtained from Cases 2 and 3 are all much lower than any observed in comparable patients in the earlier paper, and the failure of subsequent doses to produce any considerable increase of the blood concentration is very striking. Thus in all these three cases, there is evidence that the accumulation of the compound in the blood was defective to a smaller or greater degree. It is considered probable that this defective accumulation formed one of the main factors leading to these relapses.

DISCUSSION.

A search through the earlier literature shows that cases of trypanosomiasis relapsing during or shortly after treatment with Bayer 205 have been noted by previous observers also. The following instances may be quoted, and probably many other cases have been seen :—

Low (1924), records a European (Case 1) infected with *T. gambiense*, who relapsed 2 days after the fifth dose of Bayer 205. DYE (1926) mentions a native (No. 19) infected with *T. rhodesiense*, who relapsed a few days after the fifth injection had been received. CORSON (1928a), describes thirty-nine cases of Rhodesian sleeping-sickness ; of these, three are closely similar to those described above : Case 43, who relapsed 16 days after the fourth dose ; Case 57, who relapsed 9 days after the seventh dose—blood was inoculated into a rat which became infected (CORSON, 1928b) ; and Case 92, who relapsed 5 days after the third dose. This paper also contains nine other instances, in which relapses occurred 24 days to 2 months after courses of two to six doses. In a later paper CORSON (1931) describes a fourth case (Case 279) who relapsed 5 days after the

third dose ; blood containing numerous trypanosomes was inoculated into three rats but failed to infect them, although four rats, injected with blood taken before treatment began, had all become infected. Dr. H. G. CALWELL (1938, personal communication, quoted from memory), treated a European at Tabora, Tanganyika Territory, with weekly injections of antrypol (the analogous British preparation). After about three doses, the blood was found to contain numerous trypanosomes, but these disappeared under further injections of Bayer 205 and the patient was discharged a few weeks later apparently cured.

These cases seem closely similar to those of the present series ; during treatment with Bayer 205 there is a short febrile reaction, examination of the blood reveals the presence of numerous trypanosomes, which disappear again in a few days, although in many cases no further treatment has been given. The possible factors in the production of these unusual relapses have been analysed above, where it was shown that there was no evidence for increased resistance on the part of trypanosomes (in the two strains examined) ; that the phagocytes of the blood appeared normal, although jaundice occurred in Case 1, indicating some derangement in the liver ; that the potency of the specimens of drug used was probably normal ; but that the accumulation of the Bayer 205 in the blood was small in one case, and very small in the other two. It is considered that this defective accumulation of the compound was probably the main factor in producing these relapses.

The correctness of this conclusion will, it is hoped, be tested by the experience of other observers elsewhere. Meanwhile two points of practical importance may be mentioned, although both have been considered in more detail in the previous paper (HAWKING, 1940).

1. These cases show that in some patients the blood-concentration of Bayer 205 is much lower than what might have been expected in view of the number and size of the injections which they have received. In the previous paper, other examples of the same sort were described. Unless a relapse occurs, as in the present instances, such patients can be detected only by chemical estimations made on the blood. Consequently, in order to ensure that patients do in fact receive adequate treatment, it is recommended that treatment be controlled by chemical means whenever possible, *i.e.* with practically all European cases.

2. There has been much discussion of the use of Bayer 205 for prophylaxis and of the concentration in the blood which is required to prevent infection. In these patients, trypanosomes reappeared in plasma containing 4·5, 1·0 and 0·15(?) mg. per 100 ml., but since they rapidly disappeared again, although no more treatment had been given, their presence is not exactly comparable with what occurs at the initiation of a new infection. VIERTHALER and BOSELLI (1939) found that an average blood concentration of 1·3 mg. per 100 ml. was sufficient to protect rabbits against an intravenous injection of a laboratory strain of *T. brucei.*

SUMMARY.

1. Three Africans, infected with *Trypanosoma rhodesiense*, were treated with Bayer 205. During or shortly after the treatment, *i.e.*, 5, 9 and 19 days respectively after the last dose, relapses occurred, with numerous trypanosomes in the blood, accompanied by a febrile reaction. Although no further treatment was given at the time, the trypanosomes disappeared from the blood in a few days' time, and the pyrexia subsided.

2. Strains of trypanosomes were obtained from two of these patients and tested in rats. No evidence was found of any abnormal resistance to Bayer 205.

3. Chemical estimations of the concentration of Bayer 205 in the blood of these patients at the time of relapse and during subsequent treatment, showed that the accumulation of the compound in the blood was defective as compared with that of patients studied in a previous series. For reasons which are discussed, it is concluded that this defective accumulation was one of the main factors responsible for these relapses.

4. A review of the literature shows that similar relapses have been recorded by many other observers.

5. It is recommended that where possible chemical estimations should be made on the blood of patients receiving Bayer 205, in order to detect the individuals in whom such defective accumulation occurs.

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INJURIES CAUSED BY SCORPION FISH.

BY

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The sea coast of Barbados, famous for its fine bathing places has, between beaches, many coral reefs of which some adjoin the shore. Barefooted fishermen who frequent these shoals are often injured by the scorpion fish (or " lion fish," as it is known locally), which hides in the weed-covered crevices of the coral. More rarely a bather may be struck by the sharp spines of a fish which has temporarily wandered from the reef.

Natural History.—The Scorpaenidae are a large and interesting family of fish of about 30 genera and 250 species. They inhabit all seas but are especially abundant in the temperate regions of the Pacific Ocean. The species are numerous in tropical seas and they are usually fishes of singular form and bright colours, with great variation in armature. Usually they are bottom-dwelling, non-migratory fish living in the weed and rocks of the shallower waters. Most of the species are large enough to be considered as likely articles of food. In some places the flesh is reputed to be poisonous but there is no evidence to substantiate this belief. *Scorpaena plumieri* is eaten by some Barbadian fishermen but is not usually offered for sale. Many species are viviparous, the young being produced only after they have reached considerable size.

The family characteristics are an oblong body, more or less compressed dorso-ventrally, head large and usually with one or more bony ridges or stays above, which terminate in spines. Opercle with two spinous processes and preopercle usually with four or five. Mouth terminal and large, containing villiform teeth implanted on jaws, vomer and usually the palatines. Gill

openings wide, gill membranes separate and free from isthmus. Lateral line single, continuous and concurrent with back. Ventral fins thoracic, dorsal fin continuous but sometimes so deeply notched as to divide it into two parts, with eight to sixteen strong spines and as many rays. Anal fin short with three spines and five to ten soft rays.

Two species of Scorpaenidae are found around the coast of Barbados, *Scorpaena plumieri* (Bloch), and *Scorpaena grandicornis* (Cuvier and Valenciennes). Of the two, *S. plumieri* is by far the commoner : it is said reach 1 ft. in length.

GUDGER (1930) states that " entirely absent (in the West Indies) are the bottom-dwelling Scorpaenidae and Siluridae which are so dangerous to the barefooted fishermen of the Indian and Pacific Oceans." But this observation is obviously incorrect.

The question of whether the poison injected by the spines of these fish is contained in glands is much disputed; and has been since the days of ARISTOTLE, according to EVANS (1921). SONNINI, LACEPEDE and CUVIER all deny the presence of any poison gland; whilst ALMAN, BYERLEY, GUNTHER, NEWTON, PARKER and BOTTARD have admitted the presence of a gland at the root of the spine of the lesser weever (quoted by EVANS, 1921). EVANS (1921) himself describes several fish of the Scorpaenidae in which poison glands may be found.

I have dissected many of our local species and have been unable to detect any glands. There is, however, a thick mucous membrane surrounding the spines and I consider that this contains the poisonous principle which causes so much pain. On examination, this tissue shows a superficial layer of stratified epithelium with large pigmented cells underneath. In the cutis vera are found many microscopic mucus-secreting glands. Possibly these small glands in the skin covering the spines produce the poison.

If gentle pressure is applied to the median posterior third of the head over an area immediately behind the three dark spots seen in the figure (p. 229) a reflex is obtained consisting of an immediate erection of the dorsal spines. The stimulus need not necessarily be heavy, so a foot accidentally touching the back of the fish may receive one or several jagged puncture wounds. The victim immediately experiences severe pain, which becomes excruciating within a few minutes. Unless treated the pain will last for several hours and in complicated cases the patient may be in bed for several weeks.

Symptoms.—When first seen the patient is found writhing in agony, the pain being comparable to that of renal colic. He is bathed in perspiration, is pale, dyspnoeic and has severe tachycardia. Very often he is unable to tell exactly what has happened but is grasping the limb and squeezing it violently. On inspection there is marked pallor surrounding the injured areas which may show insignificant-looking abrasions. The pain is not localized—in the case of a foot the whole lower extremity is also involved. The patient, following the local custom, has usually taken large quantities of alcohol but this does not appear to have any effect in relieving the pain. Sometimes the vesicant juice of the

The Scorpion Fish.

poisonous manchineel tree, always to be found growing near the sea coast, has been applied to the wound as an antidote. Since the juice of this tree causes severe blisters wherever it touches (EARLE, 1938) it is fortunate that the punctures usually occur on areas of the body covered by thickened, cornified epithelium, otherwise these crude therapeutic measures would lead to more serious complications.

Complications.—Vomiting, accompanied by diarrhoea is frequent and after 24 hours a rash may appear. I have seen both morbilliform and scarlatiniform types. As a rule the abrasions heal readily but in certain cases abscess-formation, superficial necrosis or a low grade streptococcal lymphangitis of the affected extremity may develop. Tetanus is extremely rare but has been reported.

Treatment.—Morphine in large doses with liberal quantities of alcohol are usually administered. EVANS (1924) also advocates the injection into the puncture of a 5 per cent. solution of potassium permanganate. In my early patients these treatments were used and were partially successful ; after several hours of sleep the pain wore off.

Then one patient, a Scotsman with a great acquired resistance to alcohol, required additional measures. I infiltrated the area around the puncture with a solution of 2 per cent. procaine and 1 in 10,000 adrenaline hydrochloride. Relief came before the needle had been removed and the patient was able to go out to dinner the same evening. Since that time I have always used this form of treatment with very favourable results, obviating the use of morphine. Alcohol, which is usually requested by the patient, is also given in moderate quantities in deference to local custom.

SUMMARY.

1.—The habitat, natural history and anatomy of certain West Indian Scorpaenidae are described.

2. Injuries and their complications arising from punctures by their spines are outlined.

3. Treatment of injuries, using procaine infiltration, is indicated.

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CORRESPONDENCE.

PELLAGRA AND RED PALM OIL.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In these TRANSACTIONS XXXIV, 1, 86, Dr. CICELY WILLIAMS makes a reference to the common incidence of sore mouth and glossitis among pregnant women in West Africa. She says that red palm oil in the diet appeared to have no preventive effect: that none of the cases developed pellagra or anything like it.

This condition has been reported upon also by WRIGHT in Sierra Leone and the writer in Nigeria. Dr. KESTER, while in charge of the Lagos Maternity Hospital, Nigeria, in 1936, submitted some useful statistics. Of 737 expectant mothers attending the antenatal clinic there, in a period of one month, 116 had one or more of these signs. Most of them complained also of pruritus vulvae, which caused much discomfort and was attended by a typical rash. All the affected cases came from poor families. So common was the incidence among them Dr. KESTER found it hard to dissuade them that it was not part of normal pregnancy.

These lesions are identical in character with those observed in a more general syndrome, occurring in both sexes (affecting in particular the poorer elements, such as petty traders, unemployed, certain schools and institutions) which has already been reported upon. They differ only in degree as do the milder cases in that syndrome. There is also an apparent absence of nerve involvement, such a marked feature of the more severe type of case. The skin and mucous rashes are also far less apparent. The condition is undoubtedly pellagrinous and responds readily to autoclaved yeast or marmite. It is to be remembered however that the nervous symptoms must be taken in hand in reasonable time, when they are present.

The fact that nicotinic acid has no such dramatic effect—a point also reported upon by the writer in Nigeria (1938)—does not mean they are not pellagrinous. It has been pointed out that both adenine and riboflavine are as important as nicotinic acid and the deficiency of any one of these fundamentals would wreck the action of the whole (PETERS). Marmite contains 640 mg. per gramme of nicotinic acid.

Red palm oil does not affect the existence of this syndrome because it exists in spite of it. It is, perhaps, not sufficiently well known that red palm oil is part of the daily dietary of all Africans, who live in the palm bearing belts, which not only extend throughout so much of West Africa but to the Congo as well. This oil forms the basis of nearly all the soups or palaver sauces, an integral part of African food preparation, especially of the poor.

Red palm oil must play, however, an important determining factor in tracing the full investigation of all the signs and symptoms associated with this

syndrome, wherever it may occur. In past years much controversy has existed, not so much as to whether or not the syndrome is pellagrinous, but as to its cause being attributable to vitamin A deficiency. The absence in West Africa of any of the classically accepted features of vitamin A deficiency (such as keratomalacia) in these cases is no accident but is clearly explained by this eating of red palm oil, rich in pro-vitamin A. This same fact explains why child blindness, resultant on destructive lesions of the eye, following vitamin A deficiency is absent from West African statistical returns in these areas and why the problem of child blindness there, as a social consideration, cannot compare with those same problems in the Far East, the West Indies, etc. Defective vision, due to partial optic atrophy in association with this pellagrinous syndrome, may be very severe both in incidence and type but it rarely if ever leads to total blindness and never to destructive lesions of the eye. Blindness due to endemic disease such as smallpox or gonorrhoea is not uncommon.

It seems reasonable to state that in those countries, where not only this syndrome may exist but where also vitamin A deficiency may occur, the signs and symptoms (they include nerve degeneration) which are *common* to those countries *and* to West Africa, cannot be attributed to vitamin A deficiency. In recent years it is noteworthy that at least the sore mouth and tongue and some of the dermal rashes have been widely agreed upon as no longer attributable to vitamin A deficiency. It will be remembered that, as long ago as 1911, STANNUS viewed this syndrome as pellagrinous, of the type pellagra *sine* pellagra. Most valuable time, certainly from a nutritional view point with its implied economic considerations, has been lost over many years, through those divergencies of opinion and it seems a great pity that his original thesis has not received the attention it deserved. In later years when it became easier to follow up clinical research the range of argument was bound to widen in detail but the key was always there.

There is a further point with reference to red palm oil. The writer suggested some time ago (*Lancet*, 6th January, 1940), that it might well prove a valuable additional food source to those countries which are deficient in vitamin A and especially to those areas where standards of living might not permit the supplementary purchase of the relatively expensive (to them) fish liver oils, even if they were made available. In a recent address to the Indian Science Congress, W. R. ACKROYD has suggested that red palm oil might be blended with the common Indian food vegetable oils to assist in this way. It is interesting to note that, in this country, in time of stress, it has been found practicable to vitaminise ground nut oil. Such a method also might be made future use of overseas but this method might prove too expensive.

I am, etc.,

Parkstone,   D. FITZGERALD MOORE.
Dorset.

COMMUNICATIONS.*

CLINICAL ASPECTS OF ONCHOCERCIASIS IN THE SOUTH KAVIRONDO DISTRICT OF KENYA COLONY.

BY

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The presence of an unusually large number of cases of blindness, and of a skin condition, the local name of which amongst the natives was *nguku*, in a circumscribed area—Kodera location mainly—of South Kavirondo had been known for a number of years. It was not, however, until 1938, that the association of these cases of blindness in Kodera with onchocerciasis was established by a chain of evidence from suggestions made and work done by numerous workers.‡

The history is as follows : Several journeys had been made to Kodera location, to investigate the cases of blindness and the scaly skin condition, known by the natives as *nguku*. These journeys (which were made before it was appreciated that onchocerciasis occurred to any considerable extent

A

in East Africa) led to the conclusion that both eye and skin conditions could be accounted for by food deficiency. It had been found that the natives exported their ground-nuts and sim-sim oil out of this district, and they appeared poor as regards the number of cattle they owned.

The natives, however, maintained that both eye and skin conditions were manifestations of one disease, and that infection was caused by bathing in the
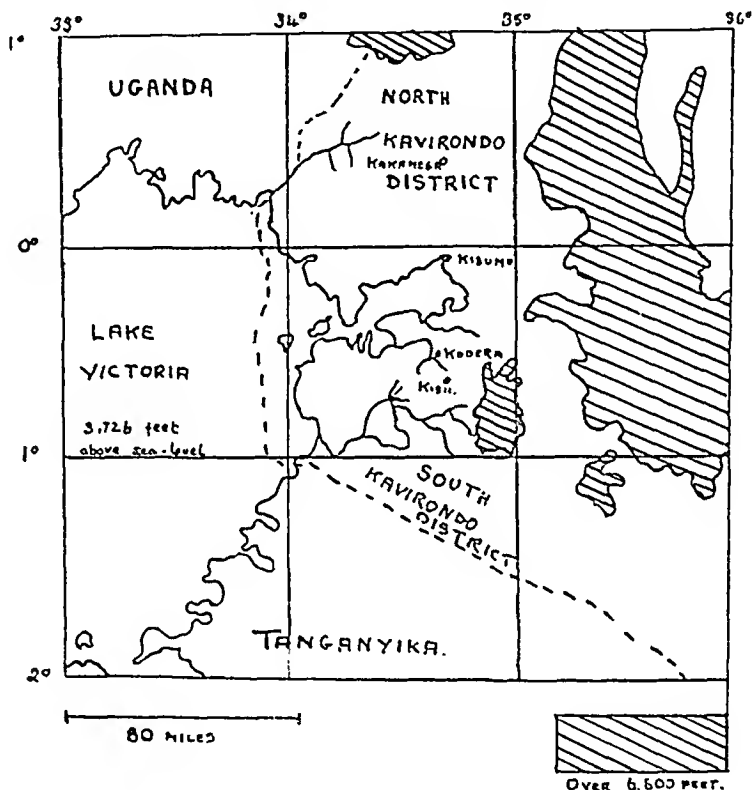
FIG. 1.
MAP OF PART OF KENYA.

local stream, the banks of which were lined by a certain variety of tree, whose branches overhung the river, and the seed-pods of which burst liberating the hairy seed into the water and infecting those bathing. They brought forward the interesting evidence that young women from neighbouring uninfected districts, when married to natives of Kodera, would become infected after having made their domicile in this district.

The adult of *Onchocerca volvulus* had been found present in a nodule removed from a native patient in Kisii Hospital by PRESTON (1935), but this had remained an isolated observation. When a tumour, removed from the head of a European in Kakamega Hospital in 1938, was found by JOBSON (1939)

to contain the adult *O. volvulus*, and when a skin-snipping survey of natives in Kakamega by HAWKING (1938) revealed a high proportion with microfilariae, it was definitely established that onchocerciasis was endemic in North Kavirondo District (see map), and HARLEY-MASON (1939) realised the significance of two cases of chronic eye trouble in Europeans of this district.

It was then recalled that there was a rocky stream near Kodera Dispensary, and the possibility of onchocerciasis being endemic in South Kavirondo District also, suggested itself. It was decided to examine the natives of Kodera location for the presence of subcutaneous nodules.

The location was visited in October, 1938, by CORMACK, who found subcutaneous tumours present in the natives attending Kodera Dispensary. Of eighteen natives with eye trouble, tumours were present in fourteen. Of a group of nineteen natives with normal eyes examined at the dispensary on the same day, only four showed tumours. Some of these tumours were subsequently excised and shown to contain the adult of *O. volvulus*. No lack of food supplies was found in the neighbourhood of the native huts and CORMACK considered that food deficiency was unlikely to be a factor accounting for the eye and skin conditions. The rocky stream just below the dispensary was visited and flies collected were subsequently identified as belonging to the genus *Simulium*. McMAHON (1940), in his fly survey, found a proportion of these infected with the larvae of *O. volvulus*. The pods referred to above were found to come from a creeper; their hairs were found capable of producing erythema on the forearm of a European, lasting for 4 to 5 days, but there was no evidence that they were the cause of the scaly skin condition.

From May till July, 1939, McMAHON investigated the entomological aspects of the disease locally, and did a skin-snipping survey. In the course of this survey, McMAHON (1940), made skin-snippings, usually of sixty natives daily, selecting a different village each day. For the first 3 days concomitant clinical observations were made on the natives submitted to the skin-snipping test by the writer of this paper, and on the subsequent 7 days of the survey by his African Hospital Assistant, John Robert s'o* Gudia, as the writer was engaged in other work. The data obtained included the name, approximate age, sex, presence of pain in the eyes, condition of sight, eye lesions, skin condition and presence of nodules. These data, of course, were correlated with presence or absence of microfilariae, as shown by McMAHON's skin-snippings. The observations so made provided data of approximate age and sex for McMAHON's human survey, and provided the writer with a list of names of natives known to have skin microfilariae, with essential clinical data concerning them, the natives being grouped according to their respective villages. From this list, by writing each month to the local Chief, the writer was able to obtain a regular supply of suitable natives for investigation of this disease.

*s'o = son of.

This paper is based on observations made in the above-quoted survey, on visits made to Kodera at other times, and on the more detailed investigation of patients subsequently brought to hospital, who were carefully examined by the writer and others* before being submitted to various methods of treatment, the efficacy of which it was our object to investigate.

Unfortunately, the military situation, with consequent reduction of our staff, forced us to discontinue these investigations at the end of August, 1939. It is regretted that the data are incomplete : it was, however, felt that the observations so far made were sufficiently interesting to be worth recording.

Clinical Observations.

1. AGE AND SEX.

The clinical data analysed in Tables I and II were collected by John Robert s/o Gudia during McMahon's survey in the infected area. from 406

TABLE I.

PERCENTAGE INFECTION ACCORDING TO SEX AND AGE DECADE.

Age Group.	Male.				Female.			
	Microfilariae in Skin-snippings.				Mirofilariae in Skin-snippings.			
	Mf. +		Mf. —		Mf. +		Mf. —	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Under 10 years ...	6	26·1	17	73·9	3	25·0	9	75·0
10–19 years ...	28	66·7	14	33·3	17	56·7	13	43·3
20–29 years ...	45	71·4	18	28·6	29	56·8	22	43·2
30–39 years ...	36	75·0	12	25·0	8	61·5	5	38·5
40 years and over...	64	68·8	29	31·2	21	67·7	10	32·3
Total ...	179	66·5	90	33·5	78	56·9	59	43·1

unselected natives. Each native was submitted to a single skin-snipping. Repeated examinations would probably have revealed the presence of microfilariae in many of those found negative on a single examination.

*I have to thank Drs. J. H. Chataway and E. P. Rigby and African Hospital Assistant Noah s/o Otembo, for their help.

The analysis of clinical data in Table I shows the correlation between age, sex and presence of microfilariae. Only the broadest conclusions can be drawn from these data. Babies and infants were not examined owing to natural prejudice on the part of their parents. With these possible exceptions, it can be said that in this area, onchocerciasis occurs at all ages and in both sexes. Under the age of 10 years, the percentage infection is definitely lower than that of older decades.

2. OCCUPATION AND DOMICILE.

The infected area lies at approximately 4,000 feet above sea-level, and in the valleys are fast-flowing, rocky streams. The natives of this area are poor, though this is probably a fortuitous accompaniment of the infection rather than its result. Their livelihood consists of cultivating crops and herding goats and cattle. All infected natives in this area were wont to visit one of the infected streams, which have been shown by McMAHON to harbour infected simulium.

On being questioned, infected natives admitted that they visited either the main river or one of the smaller streams at regular intervals. Visits were paid by most of them for bathing daily, once every three days, or once weekly. In addition, men and boys watered their cattle there daily; women went twice daily to draw water, and children would go there to play.

The paramount factor in infection is the necessity of visiting an infected stream frequently.

3. EYE LESIONS.

Eye diseases are common amongst Kavirondo natives, and form a considerable proportion of all out-patient complaints. This proportion varies according to local conditions.

The analysis of clinical data in Table II shows correlation between symptoms, etc., and presence of microfilariae in skin-snippings.

It will be observed that the proportion of natives complaining of pain in the eyes or bad sight or having abnormal eye appearance is greater in those whose skin-snippings showed microfilariae than in those in whom they were not demonstrated. This table will be referred to again below.

A considerable variety of eye complaints and abnormal eye appearances was found. The following observations, however, were made by the writer, who is not an expert on eye diseases. The sight of some of these natives of the infected area was unimpaired; others complained of failing vision, the eyes often being found unequally affected. Again, in others, vision in one or both eyes was limited to perception of light only, while in some there was complete blindness.

The only complaint in mild cases was of lachrymation and photophobia. Blepharospasm was a common feature and conjunctival hyperaemia was often present. The latter was often seen, affecting especially the bulbar conjunctivae. This was sometimes combined with diffuse superficial corneal opacities of both eyes. Other corneal changes were often present, taking the form in some cases of what appeared to be an arcus senilis (occurring also in men younger than those normally associated with this), sometimes unilateral. Sometimes the

TABLE II.

CLINICAL DATA OF CASES EXAMINED BY SKIN-SNIPPINGS FROM
JULY 1—JULY 8, 1939.

Clinical Data.	Mf. +		Mf. —	
	Number of Cases.	Per cent.	Number of Cases.	Per cent.
Poor sight or abnormal eye appearance ...	36	14·0	13	8·7
Pain in eyes	41	16·0	17	11·4
Skin nodules	85	33·1	34	22·8
Abnormal skin ...	59	23·0	21	14·1
Total number of natives examined	257		149	

It will be seen that the figures in the bottom line of Table II do not represent the sum of the figures in the upper four lines. Among the total numbers of natives examined, some individuals had no symptoms or signs, whilst others had one or a combination of more than one.

circle was incomplete, being represented only by two vertical, band-like opacities in the medial and lateral corneal margins. One variety of keratitis appeared to begin as an opacity in the lower part of the limbus (in contrast to trachoma, in which the pannus spreads from the upper part of the limbus downwards). In advanced cases, this would have spread upwards over the cornea, and the pupil would be displaced eccentrically in the direction of the opacity. In fact, such cases often showed iritis.

In one case in which an opacity was encroaching on the pupil from below, the pupil was a nearly vertical slit, reacting to light unevenly, and there was a slight iritic exudate.

In one native, aet. 36 years, there was a history of oncoming blindness for 1 year. The left eye showed a large pupil. The right eye showed an opacity occupying the lower half of the cornea, with a small slit-like pupil and white iritic exudate. Neither pupil reacted to light. Ophthalmoscopic examination of the left fundus revealed choroiditis. This man gave a history of past yaws, but not of syphilis. He had raised eyebrows, but his reflexes were normal ; his heart was not enlarged, nor was his spleen palpable.

These opacities on the inferior part of the cornea often showed an irregular line of pigment.

Cataract was by no means uncommon amongst the natives examined at Kodera.

Several of the patients appeared to be suffering from a severe degree of trachoma, which is common in Kavirondo country. What part, if any, in the eye conditions described above was played by spirochaetal infection, could not be assessed, as the Wassermann reactions of these patients were not investigated. Again, whether avitaminosis was a factor or even the main cause of some of the varieties of keratitis described above, and whether the cataracts seen were merely an exaggeration of senile cataract, could only be surmised. No examinations were made here with a slit-lamp or other special apparatus. As mentioned above, the proportion of eye complaints and eye lesions was higher amongst those Kodera natives whose skin showed microfilariae, than amongst those whose skin was negative. The view is supported that infection with onchocerciasis in a certain proportion of cases either causes lesions in the eye *per se*, or that it aggravates, or renders the eye liable to, other diseases.

4. SUBCUTANEOUS NODULES.

It will be seen from Table II, showing John Robert's figures, that subcutaneous nodules were found present in 33·1 per cent. of those showing microfilariae in their skin-nippings.

Fig. 2 shows in diagrammatic form the position of nodules in thirty-five cases of onchocerciasis admitted to Kisii Native Hospital for investigation. (All showed skin microfilariae, though not all of the thirty-five had demonstrable nodules.) The most favoured places for nodules to be found were the lower ribs and intercostal spaces, in the line of the axilla ; the anterior superior iliac spines and the crests of the ilia, the back of the head (occipital ridges and behind the ears) and over the sacrum. One nodule in this series, shown to contain the adult worm by microscopic section, was found over the right greater trochanter, and another similarly identified was found on the inner side of the left ischial tuberosity.

Amongst other sites where nodules have been found in Kodera natives are : above pinna of left ear, over right mastoid process, right malar region,

over left parotid gland, over right side of back of neck, at point of left scapula, and over right olecranon process.

Sometimes the nodules were present, at a given site, singly. Nodules, especially over the ribs and pelvic girdle, were sometimes felt as lobulated tumours, each of which was found to contain more than one nodule. Some of the nodules in a tumour were found to be small seedling nodules. In the series of thirty-five cases diagrammatically represented in Fig. 2, the nodules
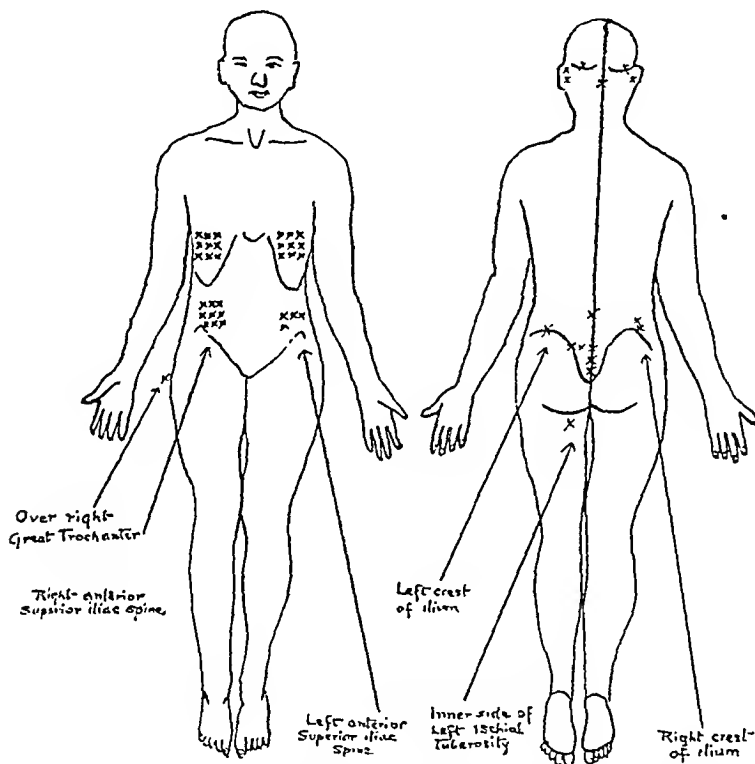
FIG. 2.—Showing position of all nodules in thirty-five natives (whose skin showed micro-filariae, and who were admitted to Kisii Hospital), superimposed on above diagrams of a single individual.

Some had no nodules ; others had several. Each cross represents one nodule.

were removed, mostly under local anaesthesia. Those over the ribs and inter-costal spaces were often found deeply attached by fibrous tissue to the intercostal membranes. In nearly every case with nodules, at least one of the nodules was incised after removal to demonstrate the adult worm. As shown in Fig. 2, nodules were always found near or over bone. Round the pelvic girdle especially there was a great tendency for the nodules to be fibrosed and sometimes even calcified ; and all stages were found from a soft nodule, showing adult worms

on incision, to calcification. To avoid the likelihood of including juxta-articular nodes, so commonly found in natives over the greater trochanter following yaws, fibrosed nodules in which worms were not demonstrated macroscopically when occurring in that situation, have not been included in Fig. 2.

Onchocercal nodules had to be distinguished from enlarged lymphatic glands, especially over the chest-wall, in the lower part of the axilla, and in cases of advanced dermal onchocerciasis to be described below.

5. SKIN LESIONS.

The minimum skin lesion seen was a rash consisting of several large papules present only over the back. The rash was pruriginous, as also was the skin over other parts of the body. The further development of the skin eruption is illustrated by the following case (R. P. CORMACK'S case) :—

Achago s/o Nyale. Male, aet. 38 years.

Pruriginous rash all over body, with exception of palms of hands ; but sparse on arms and very sparse on forearms. Not present on foot (left leg amputated below knee), sparse on leg, profuse on thigh and body. Face not attacked. Very little on neck. No nodules. Said to have been present for about 8 years. Consists of raised, firm papules, 3 to 5 mm. in diameter, which dry to form a dry scab, which can be detached leaving the base covered by epithelium.

Next, the whole thickness of the skin had increased and the skin was ichthyotic. In the most advanced stage, these changes were found to have involved all the skin below the head, and the skin had become elephantoid, *i.e.*, the subcutaneous tissue had become so much thickened that the ichthyotic skin hung in folds. This stage was illustrated by the following case :—

O.39/10. *Barna s/o Mbuyi.* Male, aet. 36 years.

Skin pruriginous, very thick and elephantoid. Distribution : whole body below head—neck ; chest ; upper arms ; forearms slightly ; trunk, front and back ; and thighs. Not on penis or scrotum. Face and forehead pale, showing papular thickenings. Groin glands both thighs enlarged ; axillary glands both sides much enlarged, soft and movable. Left supra-clavicular glands enlarged and soft.

There were also localized thickenings in the skin like fibromata. No subcutaneous nodules were palpable. An enlarged and fibrosed lymphatic gland, suspected before removal of being a nodule, was removed from the patient's right axilla.

This patient had an ulcer of his right leg, but he gave a history of yaws in childhood.

This case illustrated further features of advanced dermal onchocerciasis, *viz.*, the presence of enlarged lymphatic glands (though in this patient's case, the enlargement might have been due to yaws) and the frequent absence of any palpable nodules.

A combination of ichthyotic and elephantoid skin changes was illustrated by the following case :—

O.39/8.   *Oraga s/o Amnomo.*   Male, aet. 20 years.

Pruriginous rash.   Marked ichthyotic skin condition of back, both buttocks and thighs, with elephantoid condition of skin around left knee.   Enlarged glands present in left groin.   No nodules.

Another feature of these advanced cases of dermal onchocerciasis was the tendency, frequently noted, to the development of lipomata.

A large mass was removed under general anaesthesia from the right axilla of Miniambo s/o Muga, male, aet. 40 years, who was suffering from onchocerciasis.   The mass had been present for several years.   Laboratory report : " This appears to be a true lipoma.   I can find neither adult nor larval onchocercae." (G. L. Timms.)

In many cases, however, the skin condition appeared to have become arrested in the ichthyotic stage, and to have regressed leaving a generalised atrophy of the skin, frequently seen amongst both sexes in the older decades of the population in Kodera.

Discussion.

Hissette (1938) emphasizes the slowness of evolution of eye lesions.   He describes the following eye lesions : .(i) *Irido-Cyclitis.*   At an early stage, the eye is red, due to circum-corneal injection, and the pupil small and sometimes irregular.   This small pupil leads to pain and photophobia.   Irregularity of the pupil is due to synechiae between the posterior surface of the iris and the anterior surface of the lens.   These may lead to seclusion of the pupil, and even occlusion. Visual symptoms can be caused by larvae crossing the visual field.   Hissette describes deformations of the pupil peculiar to ocular onchocerciasis, in which the pupil is displaced downwards, rendering its long axis vertical—due to sedimentation of fibrin from pupillary exudate or dead microfilariae in the lower irido-corneal angle and subsequent contraction due to formation of fibrous tissue.

Atrophy of the iris occurs early and is shown by disappearance of the pigmented border at the margin of the pupil and an alteration in the distribution of its pigment.

(ii) *Keratitis.*   Punctate keratitis, of a kind not specific to ocular oncho-cerciasis, may be present.   Hissette describes a special variety of vascular keratitis, produced by the superficial vessels of the cornea, which he calls the pannus of onchocerciasis.   The latter, in contradistinction to trachoma, he asserts, never takes up its location in the upper one-third of the cornea.   If the remaining two-thirds of the cornea are divided into two lateral areas and a lower area, it may be stated that pannus most often starts in the two lateral areas—less frequently and later, if at all, in the lower area.   These forms of pannus progress towards the centre of the cornea and, uniting, may eventually cover two-thirds or even three-quarters of its surface.

The cornea in ocular onchocerciasis may show interstitial keratitis.

(iii) *Choroido-retinitis*. Choroidal blood-vessels are rendered visible and alterations in retinal pigment are seen, owing to migration of the latter to certain places and away from others. Changes in the optic nerve papillae are also visible.

In the above description of eye conditions among our cases, lesions were found which were probably the same as those described by HISSETTE as downward deformation of the pupil and the special variety of pannus both characteristic of ocular onchocerciasis. The severest eye lesions among our cases were often found associated with subcutaneous nodules no nearer the head than the lower intercostal spaces. In several cases of severe eye lesion, no nodules could be felt at all. It seems difficult to reconcile these findings with the assertion by STRONG (1938) that in cases in which the tumour is located at a considerable distance from the head ocular lesions are more often absent.

The photographs in STRONG's comprehensive paper (1938) show natives with multiple onchocercal nodules. In South Kavirondo, however, we have never seen such large numbers of nodules present in one individual, and this fact probably explains why onchocerciasis was for so long undiscovered here. STRONG also suggests the possibility of onchocercal infection without nodules and quotes instances of adult worms having been found free in the tissues.

The figures in Table II suggest that repeated examinations for skin microfilariae would demonstrate a higher proportion of natives from the infected district positive for microfilariae. The skin examined was from the forearm in McMAHON's skin-snipping survey, and from the upper arm when the examination was made by the native laboratory assistant at Kisii. No attempt was made to examine skin at varying distances from nodules.

STRONG (1937) describes an eosinophilia of 25 to 75 per cent. in cases of onchocerciasis. In Table III, page 245, it can be seen that eosinophilia in the sixteen cases investigated ranged from 3 to 43 per cent. (before treatment had been started) but, as no estimations were made in control natives not infected with onchocerciasis, the degree of eosinophilia was of little value in diagnosis, though of considerable interest as regards progress during the course of treatment.

STRONG (1938) found evidence of onchocercal infection in other mammals in Africa, and suggests that a species of antelope may sometimes act as a reservoir for the parasite, and that cattle might acquire infection from these under suitable conditions. No attempt has been made in South Kavirondo to investigate the existence of onchocerciasis in cattle and other animals.

INVESTIGATION OF TREATMENT.

Treatment of this disease is in the experimental stage. STRONG (1937) recommends excision of nodules under local anaesthesia. FAIRLEY (1936) recommends this, combined with injections of plasmoquine or antimony compounds.

In view of the possibility of an allergic reaction causing the ocular damage, MURGATROYD (1938) suggested desensitization by means of graduated doses of filarial antigen, a process introduced by FAIRLEY for other filarial diseases.

As we had had promising results in the treatment of one or two isolated cases of onchocerciasis by the use of a combination of fever therapy and intravenous injections of antimony sodium tartrate, following excision of nodules when present, we decided first to investigate the effectiveness of this line of treatment. Our criterion of success was to be the disappearance of microfilariae from successive skin-snippings during the course of treatment. (Possible errors in the interpretation of results, including absence of controls in this series, will bè referred to below.)

Table III shows an investigation of treatment of sixteen natives from Kodera. All patients were carefully examined on admission. Their nodules were excised under local anaesthesia the day after admission. On the same day, their skin-snippings were examined by the native laboratory assistant, Munari, who also determined the degree of eosinophilia in their blood. The former test was repeated four times before their discharge from hospital, *i.e.*, at the end of each week ; and the latter was repeated twice, *i.e.*, at the end of each fortnight. Each skin-snipping was triturated with a small quantity of saline, both being then centrifuged and a drop of the saline was placed on a slide on to which a coverslip was fixed. In Table III, under the heading of microfilariae, the denominator represents the number of coverslips examined, and the numerator the number of microfilariae counted, the complete area of each coverslip being subjected to scrutiny under the microscope. A rough quantatitive test was thus devised.*

It was decided to submit all sixteen patients to six intravenous injections of A.S.T. (antimony sodium tartrate). The first nine of the batch were to continue with a further six injections of A.S.T., making a total of twelve injections of A.S.T. received. The remaining seven of this batch were then to have six injections of T.A.B. vaccine intravenously to produce artificial pyrexia ; thus making a course of six injections of A.S.T. followed by six injections of T.A.B. vaccine intravenously. The injections were given three times a week. In the case of A.S.T., successive doses for the first three injections were $\frac{1}{2}$, $1\frac{1}{2}$, $2\frac{1}{2}$ grains respectively (5 to 10 c.c. distilled water being used to dissolve the different doses), the dose being then maintained at the last level for the remaining injections. Proportionately smaller doses were given to children. In the case of T.A.B. vaccine, which was also given intravenously, a primary injection of m3 was given and the patient's temperature recorded 4-hourly. Successive amounts of T.A.B. vaccine were prescribed so as to produce a temperature of 100° F. to 102° F. on each occasion. Local treatment was given to the eyes and to the skin of those requiring it.

*This test was devised by Dr. J. H. CHATAWAY.

TABLE III.

EFFECT OF TREATMENT.

Number.	Sex.	Age.	Condition on Admission.	Treatment.	Microfilariæ.					Eosinophil Percentage.		
O.39/1	F.	38	Eyes bad	12 Antimony sodium tartrate (A.S.T.)	2/1 (2 dead)	3/1	4/1	4/1	4/2	4	2	2
O.39/2	M.	38	Eyes bad. *Nguku*. Several nodules	12 A.S.T.	4/1	1/1	0	0	0/1	9	3	4
O.39/3	M.	42	Arcus senilis right eye. 1 nodule	12 A.S.T.	6/2	0/1	0	0	0/1	9	6	6
O.39/4	M.	48	Eyes bad	12 A.S.T.	3/1	2/1	1/1	2/1	3/1 (2 dead, 1 alive)	9	2	3
O.39/5	M.	38	Eyes bad. Early *nguku*. 5 nodules	12 A.S.T. Ran away, 1.8.39	7/1	4/1	6/1	4/1 (3 dead, 1 alive)	—	14	4	—
O.39/6	M.	Old man	3 nodules	12 A.S.T.	2/1 (2 dead)	1/1	0	2/1 (2 dead)	0/1	12	2	0
O.39/7	M.	40	Eyes bad. Early *nguku*	12 A.S.T.	2/1	1/1	0	1/1	0/1	17	13	8
O.39/8	M.	20	Eyes bad. (Trachoma.) Severe *nguku*	12 A.S.T. and eye treatment	12/1	0	0	0	0/1	43	6	4
O.39/9	M.	40	Arcus senilis both eyes. 3 nodules	6 A.S.T. and 6 T.A.B. (T.102°–103°F. with each T.A.B.)	9/2	0	0	0	1/1	21	7	12
O.39/10	M.	36	Eyes slightly affected. Severe *nguku*	2 A.S.T. 3 Anthiomaline (I.M.) and 6 T.A.B. (T. 100°–103°F, with each T.A.B.). Jeyes' baths o.m. Ran away, 31.7.39	15/2	2/1	0	0	—	11	53	—
O.39/11	F.	26	Early *nguku*. 1 nodule	6 A.S.T. and 2 T.A.B. (T. 101°–103°F. with each T.A.B.). Ran away 24.7.39	3/1	2/1	0	—	—	7	5	—
O.39/12	F.	30	Skin itch. 1 calcified nodule	6 A.S.T. and 6 T.A.B. (T. 102°F, 99·8°F., 99·0°F., for 1st 3 T.A.B. No rise with subsequent T.A.B.).	0/1	4/1	6/1	2/1	3/1 (2 dead, 1 alive)	3	4	2
O.39/13	F.	28	Eyes slightly affected. *Nguku*. 1 nodule	6 A.S.T. and 6 T.A.B. (T. raised with 3 T.A.B.s)	2/1	2/1	1/1	2/1	2/1	4	18	18
O.39/14	F.	8	Young carrier with scabies	12 A.S.T. Ung. sulphur	6/1	0	0	1/1	0/1	6	2	3
O.39/15	M.	38	Eyes bad. 1 nodule	6 A.S.T. and 3 T.A.B. (T. 101·6°–105°F. with each T.A.B.). Ran away, 24.7.39	3/1 (3 dead)	0	0	—	—	19	3	—
O.39/16	F.	38	Conjunctival hyperæmia. Early *nguku*. 2 nodules	6 A.S.T. and 6 T.A.B. (T. raised 103°F. with 1st T.A.B, slight rise with 2nd–5th, no rise with 6th)	3/1	0	1/1 (1 dead)	1/1	0/1	4	8	4

Table III records the effect of treatment on sixteen natives whose skin showed microfilariæ from July 6th till August 3rd, 1939. Examinations for mf. were made at weekly intervals; examination for eosinophil percentage at fortnightly intervals. The first columns under each of these two headings respectively represent microfilariæ and eosinophil percentage on admission, before treatment was begun.

Microfilariæ are shown as a fraction, in which the numerator represents the number of microfilariæ seen, and the denominator the total number of squares examined. Wh

Though microfilariae disappeared from the skin in some cases rapidly, in some slowly, over the period of 4 weeks under A.S.T., in some the skin was not sterilised by the full course of A.S.T. The auxiliary help of fever therapy was not clearly shown by this series of cases, although the T.A.B. injections were appreciated by most of those who had them.

The effects on two cases in this batch were outstanding. One was a very severe case of chronic dermal onchocerciasis; (for case record see O·39/10 under " Skin Lesions " above), obviously of long-standing duration. So great was the thickening of his skin that intravenous therapy was rendered difficult. With daily Jeyes' baths, he showed a rapid improvement shortly after his treatment with A.S.T. injections had begun. Owing to the difficulty of giving his injections intravenously, two of his A.S.T. injections were replaced by May & Baker's intramuscular antimony compound, anthiomaline. The second case (O.39/8) was a moderately severe dermal onchocerciasis with eye appearances suggesting trachoma. Local eye treatment combined with intravenous therapy produced great clinical improvement in both eyes and skin.

One feature brought out by the investigation of this batch was that the infected individuals who were most difficult to skin-sterilize were those with fewest symptoms and signs of the disease, e.g., O.39/12. Another feature which was suggested by this investigation was that the prognosis as regards ultimate effects of treatment in sterilizing the skin, was better in those whose blood-films showed a high degree of eosinophilia.

COMMENTARY.

Lines of investigation will have to be devised to answer the following questions :—

(1) What line of treatment will sterilize the skin of microfilariae ?

(2) Does absence from endemic area *per se* and, therefore, freedom from infected bites of simulium, cause a progressive diminution in number of skin microfilariae over a period of 4 weeks ?

(A control batch of twelve infected individuals being given intravenous injections of normal saline over 4 weeks would serve to determine this point.)

(3) Does excision of nodules alone cause a progressive diminution and eventual disappearance of skin microfilariae in individuals away from an endemic area ?

(4) Will antimony compounds as sole treatment administered (or combined with preliminary nodule excision) cause disappearance of skin microfilariae ?

If so, tri-valent or penta-valent antimony ? What compound ?

(5) Have other chemicals, *e.g.*, mercury, arsenic or bismuth compounds, or other drugs, any effect on skin microfilariae ?

Has, for instance, a combination of organic or inorganic mercury by

injection and inunction of ung. hydrarg. or ung. hydrarg. ammon.-dil. any effect ?

(6) If a chemical or drug is found that is successful in sterilizing the skin of microfilariae, what are :—

(a) Optimum dosage? for single dose and for total amount for a course.
(b) Optimum interval between doses ?
(c) Optimum method of administration ?

Will such chemical or drug be effective without removal of nodules ?

(7) Will a course of fever therapy *per se* sterilize the skin ?

(8) Will a combination of fever therapy and injections of drugs or chemicals, *e.g.*, antimony compounds, sterilize the skin ?

(9) Will an exhibition of one of the above forms of treatment combined with local non-specific treatment to skin and/or eyes render the patient symptom-free ? If so, for how long ?

(10) In those cases where microfilariae disappear from the skin during the period of 4 weeks in hospital, do they reappear at a later date in the absence of re-infection. If so, when ?

(11) Should it prove possible to sterilize the skin for a period of only a few months at a time, will that enable the disease to be controlled from a public health point of view ?

Kodera Location seems particularly suitable for the investigation of onchocerciasis, from both clinical and entomological aspects. It is only 20 miles along a motor road from the well-equipped native hospital at Kisii, which is itself outside the endemic area. Since the latter appears, from McMahon's investigations, to be circumscribed, there is the additional advantage that individuals from Kodera, after treatment in hospital, can, if desired, be transferred to a domicile outside the infected area, should continued observations over a period of months be desired. Since gold has been found in this area and processes involved necessitate visiting local streams, onchocerciasis in South Kavirondo is a matter of urgent importance to would-be European prospectors and their native employees, whose work would render them liable to infection.

SUMMARY.

Kodera Location, in the South Kavirondo district of Kenya Colony, is an endemic centre of onchocerciasis.

Steps leading to this discovery and clinical observations are described. Unusual features of dermal onchocerciasis are described, including ichthyotic and elephantoid stages, and a tendency to the development of lipomata.

Lines along which treatment may be investigated are detailed, and a description is given of some experiments with intravenous injections of antimony sodium tartrate either alone or combined with fever therapy.

The investigations so far made are incomplete.

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REPORT OF A SMALL EPIDEMIC OF HYPOVITAMINOSIS.

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To us, while stationed in a field hospital, there began to arrive one after another a number of African patients suffering from swelling, with pain and stiffness, of one or more muscle groups in a lower limb. The most obvious secondary feature of these cases was one of skin change partaking of the characters of phrynoderma, dryness and parchment texture frequently all seen together in the same patient. An apparently healthy condition of the mouths of the cases at first led us, however, to a review of the general field of myositic affections, and tended to concentrate our attention on the local and prime cause of the disability which had brought the patients to hospital.

These men limped painfully to their beds, some barely touching the ground with the toes of the affected limb, others waddling with wide stance or everted foot. Their inability or reluctance to stand or walk struck us as compounded of local stiffness, a desire to avoid pain, and a measure of either general weakness or apathy. On examining the affected limb we found it swollen, hot and tensely hard, and tender on deep pressure, in the neighbourhood of a group of muscles, usually one or both of the calves. The induration appeared mainly attributable to the condition of the muscle, and the skin, though hot and sometimes shiny, showed little or no oedema. Where slight oedema was demonstrable, the pitting tended to disappear sluggishly. No local lesions could be discovered whereby an infective agent might have gained entry, and most of the cases were afebrile. In such few cases as did show a rise of temperature, the pyrexia was attributable to malaria.

The association, however, of myositis with the presence of nematodes occurred to our minds and the discovery of the microfilariae of *Wuchereria bancrofti* in the night blood of one of the patients determined us to explore the calf of the most marked of our cases. There was revealed at this operation a congestion throughout the muscles of the calf so intense as to make it difficult to distinguish between muscle tissue and blood clot : the soleus was surrounded with blood clot within its sheath.

Having found ourselves confronted with a type of myositis so frankly haemorrhagic, we were forced to reconsider the possibility of scurvy. Miners on the Rand who have originated from fertile tropical parts of Africa are reported as subject to a scurvy unaccompanied by gum haemorrhages. As far as information is available to us in a field hospital, we believe, however, that this type of scurvy is further characterized by a cardiac hypertrophy reminiscent of beriberi. The most obvious secondary feature in our epidemic was, on the other hand, follicular hyperkeratosis.

The entities scurvy, pellagra, beriberi and vitamin A deficiency have of late become less definite and recorded epidemics of hypovitaminosis have tended to take on the character of separate diseases unto the occasion. The assignment of individual clinical features to specific missing dietetic factors is complicated not only by the incompleteness of our isolation of the vitamins themselves, but possibly also by their complex interaction with further factors, glandular or other, and finally by the varied requirements set the body of the patient in the different conditions of his life.

It is rarely that, in a diet so deficient as to cause disease, the lack of one vitamin is solely responsible for the conditions found. A complex syndrome is accordingly almost always met with and this is, we think, exemplified in the disease that we ourselves encountered. It was our object to discover what phenomena were due to hypovitaminosis of any sort ; and, in the disease picture before us, what signs and what symptoms could be attributed to the lack of any one particular vitamin in the diet the men had received.

The haemorrhagic myositis occurred in all cases in the lower limb. Most frequently the calf or both calves, but in several cases the quadriceps or hamstrings, were affected. In two cases there was a slight effusion into a knee joint, proved in one of them to be haemorrhagic. In another there was an associated haemothorax up to the level of the third intercostal space, the fluid withdrawn from which had the dark brown appearance of an old haemorrhage. Often the trouble had appeared in one group of muscles first, and later in another—perhaps of another limb. Sometimes the interval was as long as several weeks. It was indeed thus that we were early led to suspect that the disease was a general one rather than one of local infection.

The basis of the pathology of vitamin C deficiency is thought to be a failure in the maintenance of the intercellular material of the mesenchymatous tissue. Endothelium becomes permeable to the erythrocytes ; the late stages of true bone formation do not take place ; fibrous tissue undergoes degenerative changes ; red cell production in some way suffers. The classical picture is one of haemorrhages : subperiosteal haematomata, deep scleroses, serous haemorrhage, gum bleeding and hypertrophy. In our patients the haemorrhages seemed almost wholly confined to intramuscular bleeding in the lower limbs.

Further close examination of the men's gums, however, disclosed that, though spongy hypertrophy could only be found in one or two cases, many more of them bled rather easily when the gums were rubbed or pressed a little forcibly and in almost all the cases there was much blue pigment in the gums. The patients did not come under our care till some weeks after the onset of their illness and on being questioned a few of them gave us an account of an epidemic of sore mouth which they said occurred before their leg trouble : nevertheless the firm healthy appearance of the gums of the patients on arrival at our hospital gave little support to these statements. The only explanation we could give for the almost exclusive localization of the haemorrhages in the lower limb, was the possibility of an unusual stress thrown upon the legs by the wearing of puttees and the work upon which the men had been engaged.

Of the skin changes in our patients phrynoderma was the most constant. It occurred chiefly over the outer aspects of the legs and thighs, but prominent follicles were often also numerous on the outer surfaces of the forearm and in some patients extended over the abdomen and chest, while an acnoid state of the face was also frequently seen. Plugs of horny debris were obvious in the follicles on the nose of one patient, looking under a magnifying glass like a field of stubble.

Dryness and general hyperkeratinization, especially over the lower legs, was the second notable skin feature. A mosaic appearance was frequent and often accompanied by hyperpigmentation of many of the lozenge-shaped areas.

The skin over the knees, ankles, elbows and knuckles of most of the patients was very much thickened and wrinkled, and here also hyperpigmentation, and sometimes velvety texture, was observed. Some of the lower limbs had a

powdery surface while others were glazed and shiny from obvious epithelial atrophy. Over the limbs many of the hairs were seen to be broken off and others proved to be very brittle when being extracted.

Vitamin A is considered as primarily concerned in the nutrition of epithelium. In its absence atrophy and a keratinizing metaplasia are apt to occur. The phrynoderma and perhaps also the dryness of the skin which we observed might therefore have been thus accounted for, yet there was no evidence of sore mouth and only one case gave a somewhat doubtful history and evidence of night blindness. Frank xerophthalmia was also absent from our cases, though in the palpebral fissures of many of them hyperpigmentation of the sclerotic was noticed.

The parts played in the development of the skin characters by a deficiency of vitamin A, and of the PP or other factors of the vitamin B_2 complex were difficult to separate. It is widely considered that mosaic and parchment skin, and more particularly hyperpigmentation, are signs of pellagra. No alimentary disturbance, either of constipation or diarrhoea, was found. Though some of the tongues showed a fairly heavy pasty furring and others were slightly glazed there was not a single case of glossitis. Perhaps rather more frequently than usual in Africans, black pigmentation of the tongue was noticed.

A varying, but for the most part slight, degree of anaemia was found which in seven cases examined appeared to be neither definitely macrocytic nor microcytic in character. The factors contributing to this anaemia in such a disease picture as we were encountering were beyond our determination.

All of our cases showed a decided bradycardia, except one or two cases where obscuring factors were apparent. Suprasternal pulsation was marked, and an aortic diastolic murmur was present in one case. Suprasternal pulsation was however evident in more or less degree in almost all the cases. Cardiac action was generally feeble and irregularities of rhythm and tension were noticed in several of the cases.

With the object of determining whether a vitamin B_1 deficiency was involved in the circulatory picture, the blood pressures of twelve of our cases and of four controls were taken. One c.c. of liquor adrenalini hydrochloridi (B.P.) was then given each of them hypodermically and their blood pressures read again 5 minutes afterwards. In the controls it was found that the diastolic pressure was raised in three cases out of four, whereas the hypovitaminic cases suffered a fall of diastolic pressure in all but two cases. In three of them the diastolic murmur continued to be heard after the removal of the cuff of the sphygmomanometer, within 15 minutes of the administration of the adrenalin. One of these three patients had marked suprasternal pulsation and an aortic diastolic murmur.

The patellar reflex of our cases tended to be excessive, though normal, and even diminished response was met with in some cases. Anaesthesia was only found in one case—down the medial aspect of the shin of one leg.

No albuminuria was found in any of the cases but one man showed a few red blood cells in his urine.

Tables I, II and III give an analysis of our cases; Table IV, the red cell measurements of seven of the cases; and Table V, the detailed mean tone pressure findings.

All the patients belonged to the Jaluo tribe and had been recruited from the neighbourhood of Kisumu on the borders of Lake Victoria Nyanza. This country is one of high rainfall, and exceptional fertility. Careful questioning of the men elicited that they had been accustomed in their own homes to a varied diet including an abundance of fresh fruit and vegetables as well as of milk, eggs and fish. It is interesting to note that this tribe is particularly addicted to a form of spinach known among them as *dek*. The patients all asserted that they used this green vegetable regularly and frequently, some of them indeed daily. Preparation of it for food consisted of boiling it in ghee made from milk.

Shortly after recruitment these men were transferred to a dry, arid, semi-desert country, to do work on roads and other heavy manual labour. The ration issued to them differed in a marked degree from the diet to which they had hitherto been accustomed, consisting only of beans, unmilled rice, maize meal, dates and jaggery. Meat was issued during the later weeks. They asserted that lime juice was received by them on only 3 days during the 4 months of their stay in the area. One man belonging to another group said he had it more frequently. The symptoms described began to develop towards the end of their period of residence and some 3 to 4 months after they had left their own country.

An outbreak in 1928 of scurvy among askaris in a somewhat similar station in the same district has been recorded*. On that occasion the freedom from attack of the surrounding civil population was ascribed to the consumption of milk from their cattle.

SUMMARY.

1. A small epidemic believed to be due to a complex vitamin deficiency is described.

2. The cause of disability and hospitalization was a haemorrhagic myositis of the lower limb.

3. There were associated skin changes which are described.

4. Bradycardia and exaggerated patellar reflexes were noted.

5. Gums, which at first appeared noticeably healthy, proved on further investigation to bleed somewhat readily.

6. A rather outstanding change in the diet of the patients 3 to 4 months previous to the onset of the disease is described.

* *Kenya & East African med. J.* (1929). **5**, 350-356.

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Tables I, II and III give an analysis of our cases; Table IV, the red cell measurements of seven of the cases; and Table V, the detailed mean tone pressure findings.

All the patients belonged to the Jaluo tribe and had been recruited from the neighbourhood of Kisumu on the borders of Lake Victoria Nyanza. This country is one of high rainfall, and exceptional fertility. Careful questioning of the men elicited that they had been accustomed in their own homes to a varied diet including an abundance of fresh fruit and vegetables as well as of milk, eggs and fish. It is interesting to note that this tribe is particularly addicted to a form of spinach known among them as *dek*. The patients all asserted that they used this green vegetable regularly and frequently, some of them indeed daily. Preparation of it for food consisted of boiling it in ghee made from milk.

Shortly after recruitment these men were transferred to a dry, arid, semi-desert country, to do work on roads and other heavy manual labour. The ration issued to them differed in a marked degree from the diet to which they had hitherto been accustomed, consisting only of beans, unmilled rice, maize meal, dates and jaggery. Meat was issued during the later weeks. They asserted that lime juice was received by them on only 3 days during the 4 months of their stay in the area. One man belonging to another group said he had it more frequently. The symptoms described began to develop towards the end of their period of residence and some 3 to 4 months after they had left their own country.

An outbreak in 1928 of scurvy among askaris in a somewhat similar station in the same district has been recorded*. On that occasion the freedom from attack of the surrounding civil population was ascribed to the consumption of milk from their cattle.

SUMMARY.

1. A small epidemic believed to be due to a complex vitamin deficiency is described.

2. The cause of disability and hospitalization was a haemorrhagic myositis of the lower limb.

3. There were associated skin changes which are described.

4. Bradycardia and exaggerated patellar reflexes were noted.

5. Gums, which at first appeared noticeably healthy, proved on further investigation to bleed somewhat readily.

6. A rather outstanding change in the diet of the patients 3 to 4 months previous to the onset of the disease is described.

* *Kenya & East African med. J.* (1929). **5**, 350-356.

TABLE I.

MUSCLE INVOLVEMENT.

Case Number.	Week of Disease.	Days in Hospital.	Sites and Periods of the Limb Affections Suffered.	Maximum Difference between Circumferences of Legs. (inches).	Extent of Swelling along the Leg.
1	12th	39	(1) Left calf throughout illness	1	Whole of left leg
			(2) Right calf last four weeks	—	—
			(3) Slight effusion into left knee joint found on examination		—
			(4) Pain and tenderness over left olecranon found on examination	—	—
2	11th	39	(1) Left calf throughout illness	—	
			(2) Right calf last three weeks	$\frac{1}{2}$	Middle third of leg
3	8th	37	(1) Right calf and medial aspect of tibia throughout	1	Middle third of leg
			(2) Left thigh (hamstrings) last four weeks	1	
4	8th	32	(1) Left calf throughout	$1\frac{1}{4}$	Whole of leg and lower third of thigh
			(2) All rib cartilages found prominent and tender		
5	7th	26	(1) Left thigh (quadriceps) subsided before examination	—	—
			(2) Slight effusion into left knee found on examination		
6	7th	24	(1) Right calf throughout	$\frac{1}{2}$	Whole leg to just above knee
7	6th	24	(1) Right calf	$\frac{1}{4}$	Lower two-thirds of leg
			(2) Left calf last week		
8	5th	22	(1) Left calf (Exploratory operation)	2	Whole lower leg
9	3rd	10	(1) Right thigh (quadriceps) throughout	$\frac{1}{4}$	Lower third of thigh to calf
			(2) Left thigh (quadriceps) found on examination		
10	2nd	4	(1) Right calf throughout	1	Lower third of thigh and leg
			(2) Left calf last week		
11	3rd	4	(1) Left calf	$\frac{1}{4}$	Whole lower leg
12	4th	4	(1) Left calf throughout		Whole lower leg
			(2) Right calf last two weeks	1	
13	5th	4	(1) Right calf throughout	$1\frac{1}{2}$	Lower two-thirds of leg
			(2) Left calf last two days	—	

TABLE I—*(continued)*.

Case Number.	Week of Disease.	Days in Hospital.	Sites and Periods of the Limb Affections Suffered.	Maximum Difference between Circumferences of Legs. (inches).	Extent of Swelling along the Leg.
14	2nd	2	(1) Right leg—indefinite and uncertain for two months (2) Left calf induration and pain ten days	2	Middle thigh to lower third of leg
15	6th	1	(1) Right thigh throughout (2) Left haemothorax (and prominent rib cartilage, possibly also throughout)	1	Above and below knee
16	12th	1	(1) Left leg throughout illness	1½	Above knee to ankle
17	1st	1	Left leg throughout illness	1	Above knee to ankle
18	5th	26	(1) Right shin throughout illness. Appeared rather to be periosteal. No bony change in skiagram	½	Lower leg
19	1st	1	(1) Right calf throughout (2) Left calf throughout	Nil	Both lower legs
20	3rd	1	(1) Right calf	1	Lower leg
21	2nd	1	(1) Right leg from ankle to quadriceps for seven days (2) Right arm for seven days	—	Ankle to lower third of thigh
22	6th	1	(1) Right calf for four weeks	1	Lower leg
23	7th	1	(1) Left calf throughout (2) Right calf four weeks	½	Calf to thigh in both cases
24	4th	1	(1) Right calf throughout (2) Left calf two weeks	—	Calf to middle third of hamstrings in both cases
25	Indef.	1	(1) Right leg and ankle, indefinite (2) Left hamstrings	—	
26	4th	1	(1) Right calf four weeks	½	Lower leg
27	2nd	1	(1) Left calf throughout	1	Lower leg
28	4th	1	(1) Right calf throughout	1	Lower leg
29	4th	1	(1) Right calf throughout	1½	Leg to middle third of thigh
30	12th	1	(1) Right calf throughout	1	Lower leg
31	4th	1	(1) Right calf throughout	1	Lower leg

TABLE II.

CONDITION OF SKIN AND MOUTH.

Case Number.	Week of Disease.	Days in Hospital.	Skin.	Mouth.
1	12th	39	Marked phrynoderma over legs. Many hair stumps. Some poorly elastic oedema along the left shin	Healthy
2	11th	39	Slight general phrynoderma with broken hairs. Phrynoderma on one leg and on the other parchment skin. Acnoid face. Pigment at palpebral fissures. Slight tinea on chest	Tongue shows much black pigment
3	8th	37	Phrynoderma. Many follicles plugged. Many hairs on trunk and limbs broken. Pityriasis on chest. Parchment skin of one leg and powdery surface of other leg. Some skin hyperpigmentation. Pigment at palpebral fissures with doubtful Bitôt's spots	Tongue slightly glazed
4	8th	32	Parchment skin over left leg and powdery skin over right leg. Widespread phrynoderma and considerable, scattered, hyperpigmentation. Pigment at palpebral fissures	Dry lips. Gums blue and showing spongy hypertrophy and ulcerated margins which bleed easily
5	7th	26	General phrynoderma with many hair stumps. Powdery and parchment skin over legs	Healthy
6	7th	24	Parchment skin and hyperpigmentation over legs. General phrynoderma. Acnoid face	Healthy
7	6th	24	Phrynoderma over legs and arms. Acnoid face	Healthy
8	5th	22	Phrynoderma over both legs and thighs. Parchment skin over knees. Powdery skin over abdomen	Tongue furred. Gums bleeding
9	3rd	10	Follicular plugs resembling stubble on nose. Acnoid on cheeks. Parchment skin of legs. General phrynoderma	Healthy
10	2nd	4	General phrynoderma. Acnoid face	White furring of tongue. Teeth dirty and deficient in enamel. Gums bleed when rubbed
11	3rd	4	Slight general phrynoderma. Some hair stumps. Patchy hyperpigmentation over legs. Pigment at palpebral fissures	Healthy
12	4th	4	Phrynoderma and broken hairs over limbs. Pigmentation at palpebral fissures	Healthy
13	2nd	2	Parchment skin over legs. Stubble like follicular plugs and broken hairs over outer surfaces of arms. Acnoid cheeks	Tongue shows much black pigment
14	5th	4	Phrynoderma and parchment skin	Tongue shows much black pigment
15	6th	1	Phrynoderma over legs and thighs and slightly over arms	Lips very dry. Tongue pale and glazed

TABLE II—*(continued)*.

Case Number.	Week of Disease.	Days in Hospital.	Skin.	Mouth.
16	12th	1	Marked parchment skin over legs. Slight phrynoderma	One or two teeth with surrounding slightly unhealthy gums
17	1st	1	Glazed tissue paper skin over legs. Parchment skin and velvety textures over joints. General phrynoderma, very noticeable on chest	Tongue furred. Gums blue and somewhat hypertrophied and bleeding at slightest touch
18	5th	26	Alternating slight phrynoderma and parchmenting over legs. Velvet texture over joints	Healthy
19	1st	1	Phrynoderma of thighs	Gums bleed slightly if pressed hard
20	3rd	1	Slight phrynoderma and parchmenting	Healthy
21	2nd	1	Slight phrynoderma over legs and arms. Slight parchmenting of legs. Velvet texture of the thickened skin over joints	Gums spongy and tender and bleed easily
22	6th	1	Much phrynoderma over thighs. Skin over legs glazed and hyperpigmented. Velvet texture over joints	Gums spongy and bleed easily
23	7th	1	Many broken hairs. Slight phrynoderma over thighs and arms, and glazing of skin over legs	Healthy
24	4th	1	Dry thickened skin with close set patches of hyperpigmentation over both legs	Slightly blue and hypertrophied gums that bleed easily
25	Indefinite	1	Much phrynoderma over legs. Hyperpigmentation at palpebral fissures	Healthy
26	4th	1	Broken hair stumps over legs. Velvet texture seen on knees	Healthy
27	2nd	1	Phrynoderma over thighs. Hyperpigmentation at palpebral fissures	Blue, glazed, hypertrophied gums that bleed easily
28	4th	1	Phrynoderma over thighs. Hyperpigmentation at palpebral fissures	Gums hypertrophied and bleed copiously when pressed
29	4th	1	Phrynoderma and broken hairs. Hyperpigmentation at palpebral fissures	Old gingivitis
30	12th	1	Phrynoderma over legs and arms. Follicular plugs over nose. Velvet texture over knees. Hyperpigmentation at palpebral fissures	Blue gums, marked gingivitis round three teeth. Pigmented tip of tongue
31	4th	1	Phrynoderma over legs and chest	Gums blue, ulcerated and bleed copiously when pressed. Tongue tremulous

TABLE III.
NERVE, CIRCULATORY AND OTHER FEATURES.

Case Number.	Week of Disease.	Days in Hospital.	Patellar reflexes.		Anaesthesia.	Pulse Rate.	Heart.	Haemoglobin percentage.	Parasites.	Other Notes.
			Left.	Right.						
1	12th	39	++	Normal	—	54	Soft apical murmur	50	*Plasmodium* and *Trichuris*	Enlarged spleen. Skiagram of bones normal
2	11th	39	+	+	Along inner aspect of left shin	50	Suprasternal pulsation. Feeble cardiac action	80	*Plasmodium*	—
3	8th	37	+	+	—	62	Feeble cardiac action. Marked suprasternal pulsation. Aortic diastolic murmur	80		After adrenalin a diastolie murmur discernible at elbow persisted after removal of pressure
4	8th	32	++	+	—	56	Heart slightly enlarged. Suprasternal pulsation	55	*Ancylostoma*	
5	7th	26	+	+	—	60	—	80	*Taenia saginata*	Three attacks of epistaxis
6	7th	24	Normal	Normal	—	66	—	100	—	
7	6th	24	Normal	Normal	—	64	—	80	—	A few red blood cells in urine
8	5th	22	?	+	—	76	—	90	—	
9	3rd	10	Slight	Slight	—	72	—	80	*Trichuris*	Operation on left leg
10	2nd	4	+	+	—	60	—	80	—	Chronic malaria. Enlarged spleen
11	3rd	4	++	++	—	58	Feeble cardiac action	80	*Taenia saginata*	
12	4th	4	++	++	—	54		95	—	
13	5th	4	Normal	Normal	—	54	—	50	—	
14	2nd	2	+	+	—	64	—	90	*W. bancrofti* and *Ascaris*	
15	6th	1	++	++	—	124	—	90	*Plasmodium*	Haemothorax. Discrete glands in groins

Table III—(continued).

Case Number.	Week of Disease.	Days in Hospital.	Patellar reflexes. Left.	Patellar reflexes. Right.	Anaesthesia.	Pulse Rate.	Heart.	Haemoglobin percentage.	Parasites.	Other Notes.
16	12th	1	+	+	—	100	—	90	Plasmodium	Malaria
17	1st	1	++	++	—	64	Irregular		—	Left femoral glands enlarged
18	5th	1	Slight	Normal	—	60	—	100	Taenia saginata	No bony changes in skiagrams
19	1st	1	Slight	Slight	—	72	—	95	—	
20	3rd	1	Slight	Normal	—	48	Very feeble action and irregular tension pulse	95	—	Spleen enlarged. Slight ulcer on shin with history of trauma
21	2nd	1	+	Normal	—	60	Small volume pulse. Suprasternal pulsation	100	—	
22	6th	1	++	++	—	64	Tachycardia on least exertion	95	—	—
23	7th	1	+	+	—	80	Very feeble action	85	Plasmodium	—
24	4th	1	++	++	—	60	—	100	—	Few pus cells in urine
25	?	1	+	+	—	66	Feeble action	100	—	—
26	4th	1	Slight	Normal	—	56	Very feeble action	90	—	—
27	2nd	1	Normal	Normal	—	60	—	90	Taenia saginata	
28	4th	1	++	++	—	64	—	90	Taenia saginata	
29	1th	1	++	++	—	66	Feeble action	50	Taenia saginata	
30	12th	1	++	++	—	72	Very feeble action	90	Plasmodium	
31	4th	1	+	+	—	68	Slight suprasternal pulsation	80	Taenia saginata	

TABLE III.

BLOOD, CIRCULATORY AND OTHER FEATURES.

Case Number.	Week of Disease.	Days in Hospital.	Patellar reflexes. Left.	Patellar reflexes. Right.	Anaesthesia.	Pulse Rate.	Heart.	Haemoglobin percentage.	Parasites.	Other Notes.
1	12th	40	++	Normal	..	64	Soft apical murmur	60	Plasmodium and Trichina	Enlarged spleen. Skin. state of bones normal
2	11th	40	+	+	About inner aspect of left side	60	Suppurated pulsation. Feeble cardiac action	80	Plasmodium	..
3	8th	47	+	+	..	82	Feeble cardiac action. Marked suprasternal pulsation. Aortic diastole murmur	80	..	After adrenalin a drastic tetanus discernible at elbow persisted after removal of pressure
4	8th	42	+	+	..	60	Heart slightly enlarged. Suprasternal pulsation	60	Anchylostoma	Three attacks of epistaxis
5	7th	28	+	+	..	60	..	80	Taenia saginata	..
6	7th	21	Normal	Normal	..	64	..	100	Trichina	A few red blood cells in urine
7	6th	21	Normal	Normal	..	70	..	80	Trichina	Operation on left leg
8	5th	23	?	+	..	72	..	80	..	Chronic malaria. Enlarged spleen
9	8th	10	Slight	Slight	..	60	Feeble cardiac action	80	Taenia saginata	..
10	2nd	—	+	+	..	60	..	90	Anchylostoma and Ascaris	..
11	3rd	—	++	++	..	64	..	60	Plasmodium	..
12	4th	—	++	Normal	..	64	..	80	..	..
13	6th	—	Normal	Normal	..	64	..	80	..	..
14	2nd	5	+	+	..	64	..	90	..	Rheumatism. Enlarged spleen in groin
15	6th	—	++	++	..	124	..	90	..	..

SULPHANILAMIDE IN THE TREATMENT OF ULCERATIVE GRANULOMA.

BY

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The report by Ross (1939) of a case of ulcerative granuloma successfully treated with M. & B. 693 (2-sulphanilylaminopyridine) has encouraged me to give an account of a number of cases in the treatment of which sulphanilamide derivatives have played a more or less important rôle.

CASE HISTORIES.

The six cases described below are arranged in chronological order. In this way the " trial and error " technique, which led to the good results in Cases 4, 5 and 6, is indicated.

CASE 1.

E. P., negro, labourer, aged 30. First seen on 3rd April, 1939, when he complained of ulceration of the penis of 8 weeks' duration. This had been unsuccessfully treated with herbal remedies.

On Examination.—A well-built man. No abnormalities in cardio-vascular, pulmonary or central nervous systems.

The glans penis, coronal sulcus and part of the prepuce were covered with an ulcerating granuloma of the dry nodular type (RAJAM, 1937). Paraphimosis was present. There was no inguinal adenitis.

Laboratory Findings.—Donovan bodies in scrapings from ulcer.

Treatment and Progress.—On 3.4.39, patient was given M. & B. 693 with instructions to take 2·0 grammes on that day, 2·5 grammes on 4.4.39, and 0·5 gramme on 5.4.39; he was also given tartar emetic (0·03 gramme) intravenously. On 5.4.39, he reported violent vomiting and arthralgia following the emetic injection: patient refused a second emetic injection: lotio rubra was

TABLE IV.

RED CELL DIAMETERS.

Number.	Macrocytes.	Mean Diameter of all Cells.	Microcytes.
	μ	μ	μ
1	7·8	7·7	7·5
2	8·0	7·5	7·4
3	8·5	7·6	7·4
4	7·8	7·6	7·0
5	7·9	7·3	7·1
6	7·8	7·5	7·4
7	7·8	7·7	7·5

TABLE V.

EFFECT OF ADRENALIN ON DIASTOLIC PRESSURE.

Case Number.	Before adrenalin.	Five minutes after adrenalin.	Change.	Fifteen minutes after adrenalin.
3	40	Nil	—40	Nil
7	104	86	—18	
8	70	80	—10	
20	78	78	Nil	
22	78	60	—18	
23	88	68	—20	Nil
24	54	50	— 4	
25	75	72	— 3	
29	88	75	—13	
30	96	78	—18	
31	80	72	— 8	Nil
Unnumbered	88	74	—14	
Control A	68	72	+ 4	
B	60	56	— 4	
C	54	68	+14	
D	50	80	+30	

She had attended a venereal diseases clinic and had received a course of intravenous injections (? tartar emetic). The condition had shown slight remissions, but no permanent cure had been obtained.

On Examination.—A sturdily-built female. No lesions of cardio-vascular, pulmonary, alimentary or central nervous systems. Large ulcerating granuloma of right labium major (which was oedematous), extending back almost to the anus.

Laboratory Findings.—Donovan bodies in ulcer scrapings.

Treatment and Progress.—On **2.6.39**, the patient was given M. & B. 693, 2·5 grammes daily for 5 days. Seen on **7.6.39**, no improvement could be observed : neoprontosil, 5 c.c. of a 5 per cent. aqueous solution, was injected intramuscularly. On **12.6.39**, the patient was given a further injection of neoprontosil (5 c.c.) and M. & B. 693, 2·0 grammes daily for 5 days was also given. Slight healing was recorded on **14.6.39**, but patient complained of insomnia. 5 c.c. of neoprontosil was given. Bi-weekly injections of neoprontosil, together with M. & B. 693 orally was given for a further 3 weeks but no marked improvement occurred and the patient finally defaulted.

Observation.

Massive dosage of mixed sulphanilamide compounds had scarcely any effect on the old-standing lesion. The past history suggests that the case was also resistant to tartar emetic. I consider that this case might have benefited by a course of fuadin together with M. & B. 693. The insomnia noted was a side-effect of sulphanilamide therapy.

CASE 3.

W. F. O., English accountant, aged 30, was first seen on 30th June, 1939. There was a past history of gonorrhoea in 1935 and inguinal adentitis in 1937. A Frei test performed during this latter condition was negative. On **3.6.39**, the patient had coitus with a negress and developed a small sore on the coronary sulcus on **11.6.39**. This was followed later by two more sores—one on the fraenum and the other on the coronary sulcus. The patient obtained outside advice and was given two intramuscular injections of bivatol (carboxyethyl-β-methylnonoate of bismuth) 3 c.c. and three intramuscular injections of solusalvarsan (sodium 3 : 4'-diacetylamino-4-hydroxyarsenobenzene-2'-glycollate).

The lesions continued to increase in size and became painful and the patient then consulted me.

On Examination.—A healthy man of fair physique. No abnormalities detected in cardio-vascular, pulmonary, alimentary, or central nervous systems. In the left groin were scars of old sinuses. There was no inguinal lymphatic enlargement. On the dorsal aspect of the coronal sulcus, at either side of the midline, were granulomata, that on the left side measuring 0·5 cm. in diameter

given as a local treatment. On **11.4.39,** the lesion, though no smaller, was drier and less offensive : M. & B. 693, 2·0 grammes daily for 5 days, was given. The lesion was worse on **20.4.39** and another injection of tartar emetic was given. On **22.4.39,** no improvement having been noted, the patient was given neoprontosil (disodium 4-sulphonamidophenyl-2-azo-7-acetylamino-1-hydroxynaphthalene-3 : 6-disulphonate), 5 c.c. of 5 per cent. aqueous solution, intramuscularly. The lesion was again dry and showed some signs of healing on **25.4.39,** when neoprontosil, 5 c.c., was again given. Neoprontosil 5 c.c., together with M. & B. 693, 2·5 grammes daily for 4 days was given on **2.5.39** when drying and reduction in size of the lesion was noted. On **6.5.39,** no further improvement was noted : patient was given neoprontosil, 5 c.c. and M. & B. 693, 2·5 grammes daily for 4 days.

On **6.5.39,** no further improvement had been noted : patient was given neoprontosil, 5 c.c. and M. & B. 693, 2·5 grammes daily for 4 days. On **8.5.39,** no improvement was noted ; neoprontosil, 5 c.c., was injected and scarlet-red sulphonate ointment (azobenzenedisulphonic acid-azo-β-naphthol) given as a local application. Seen on **10.5.39** and no improvement noted, patient was given an intravenous injection of antimony and sodium tartrate (0·065 gramme in solution). On **13.5.39,** distinct improvement was noted and a second injection of antimony and sodium tartrate, similar to the first was given. Improvement was again noted on **16.5.39,** when neoprontosil, 5 c.c., was again injected (no more antimony salt being available). Improvement was maintained and on **19.5.39,** M. & B. 693, 2.0 grammes daily, for 4 days, was given. On **23.5.39, 26.5.39, 3.6.39, 5.6.39** and **15.6.39,** neoprontosil, 5 c.c., was injected ; but after the last date no improvement occurred and subsequently the patient left the country.

Observations.

This case, in which oedema of the penis, with paraphimosis, existed was the type in which antimony resistance frequently develops (BAYLEY, 1937 ; EARLE, 1938). In such cases, the lesion continues to spread in spite of antimony therapy. The fact that no spread occurred, coupled with the fact that improvement occurred when antimony was given (unfortunately, supplies of antimony were at that time delayed) led me to think that had regular and combined dosage of antimony and sulphanilamide been given, the result would have been much more gratifying. As it was, the fact that no spread of the lesion occurred suggests that sulphanilamide used alone, whilst not bringing about cure, exerted an inhibitory effect, preventing extension of the granuloma.

CASE 2.

E. M., negress, aged 28. First seen on 2nd June, 1939, when she gave a history of pudendal and perineal ulceration commencing about a year previously.

She had attended a venereal diseases clinic and had received a course of intravenous injections (? tartar emetic). The condition had shown slight remissions, but no permanent cure had been obtained.

On Examination.—A sturdily-built female. No lesions of cardio-vascular, pulmonary, alimentary or central nervous systems. Large ulcerating granuloma of right labium major (which was oedematous), extending back almost to the anus.

Laboratory Findings.—Donovan bodies in ulcer scrapings.

Treatment and Progress.—On **2.6.39**, the patient was given M. & B. 693, 2·5 grammes daily for 5 days. Seen on **7.6.39**, no improvement could be observed : neoprontosil, 5 c.c. of a 5 per cent. aqueous solution, was injected intramuscularly. On **12.6.39**, the patient was given a further injection of neoprontosil (5 c.c.) and M. & B. 693, 2·0 grammes daily for 5 days was also given. Slight healing was recorded on **14.6.39**, but patient complained of insomnia. 5 c.c. of neoprontosil was given. Bi-weekly injections of neoprontosil, together with M. & B. 693 orally was given for a further 3 weeks but no marked improvement occurred and the patient finally defaulted.

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Massive dosage of mixed sulphanilamide compounds had scarcely any effect on the old-standing lesion. The past history suggests that the case was also resistant to tartar emetic. I consider that this case might have benefited by a course of fuadin together with M. & B. 693. The insomnia noted was a side-effect of sulphanilamide therapy.

CASE 3.

W. F. O., English accountant, aged 30, was first seen on 30th June, 1939. There was a past history of gonorrhoea in 1935 and inguinal adentitis in 1937. A Frei test performed during this latter condition was negative. On **3.6.39**, the patient had coitus with a negress and developed a small sore on the coronary sulcus on **11.6.39**. This was followed later by two more sores—one on the fraenum and the other on the coronary sulcus. The patient obtained outside advice and was given two intramuscular injections of bivatol (carboxyethyl-β-methylnonoate of bismuth) 3 c.c. and three intramuscular injections of solu-salvarsan (sodium 3 : 4′-diacetylamino-4-hydroxyarsenobenzene-2′-glycollate).

The lesions continued to increase in size and became painful and the patient then consulted me.

On Examination.—A healthy man of fair physique. No abnormalities detected in cardio-vascular, pulmonary, alimentary, or central nervous systems. In the left groin were scars of old sinuses. There was no inguinal lymphatic enlargement. On the dorsal aspect of the coronal sulcus, at either side of the midline, were granulomata, that on the left side measuring 0·5 cm. in diameter.

and that on the right, 1·0 cm. A third granuloma was seen on the right side of the fraenum extending along the prepuce. The lesions were of the ulcus molle type (RAJAM, 1937), and were tender on palpation.

Laboratory Findings.—Wassermann reaction negative. Scrapings from the ulcers showed Donovan bodies but no *Treponema pallidum.*

Treatment and Progress.—Between **30.6.39** and **20.7.39,** the patient was given eleven injections of fuadin (sodium antimony-III-*bis*-catecholdisulphonate of sodium in a 6·3 per cent. aqueous solution) totalling 53 c.c. The left-sided lesion appeared to get smaller, but the other two increased in size and the prepuce became oedematous. No enlargement of the inguinal lymphatics occurred. An erosive balanitis developed on the left side of the fraenum, which did not respond to any of the antiseptic measures recommended by HARRISON (1936) *e.g.* hydrogen peroxide, salvarsan solution, *bis*-hydroxybromophenyl sulphide. The last three fuadin injections had given rise to intense arthralgia and myalgia so that suspension of the fuadin course was necessary.

On **22.7.39,** the patient was given a course of M. & B. 693, 3·0 grammes daily, for 4 days. This produced headache, nausea and epigastric pain. On **27.7.39,** the granulomata and balanitis had completely disappeared, leaving no scarring. Periodic examinations up to the present time (**18.11.39**) have shown no return of the condition.

Observations.

The dramatic termination of this case was undoubtedly due to the M. & B. 693 ; the action of this drug resembling that in the case described by Ross (1939) where cure was obtained in 13 days. It must be remembered, however, that the course of fuadin may have rendered the lesions more amenable to the M. & B. 693.

Here again the oedematous penis and sluggish response to fuadin pointed to a possibility of developing antimony-resistance (EARLE, 1938), a condition which was obviated by the use of the M. & B. 693.

CASE · 4.

O. O., negro labourer, aged 28, first seen on 10th August, 1939, when he complained of an ulcer on the prepuce and a swelling in the right groin.

On Examination.—Well-built, muscular subject. No lesions found in the cardio-vascular, pulmonary, alimentary or central nervous systems. In the right groin was a bubo, the size of a walnut, non-fluctuant and unattached to skin or deep structures. On the inner surface of the prepuce, which was oedematous, was an ulcer, 2 cm. in diameter.

Laboratory Findings.—Scrapings of the ulcer showed Donovan bodies. Frei test positive.

Case 8

R. R., aged seven, was admitted to the hospital
August, 1868, suffering in sickness in which he had suffered from
his infancy. Upon examining the state of the parts an ulcer
of the left cornea and corneal region was observed. The lesion had
for over a month, and the patient had attended a general dispensary,
received a course of injections (been emitted but these had
producing no apparent amelioration he had detailed and
...and herbal remedies. The lesion

and that on the right, 1·0 cm.   A third granuloma was seen on the right side of the fraenum extending along the prepuce.   The lesions were of the ulcus molle type (RAJAM, 1937), and were tender on palpation.

Laboratory Findings.—Wassermann reaction negative.   Scrapings from the ulcers showed Donovan bodies but no *Treponema pallidum*.

Treatment and Progress.—Between **30.6.39** and **20.7.39,** the patient was given eleven injections of fuadin (sodium antimony-III-*bis*-catecholdisulphonate of sodium in a 6·3 per cent. aqueous solution) totalling 53 c.c.   The left-sided lesion appeared to get smaller, but the other two increased in size and the prepuce became oedematous.   No enlargement of the inguinal lymphatics occurred.   An erosive balanitis developed on the left side of the fraenum, which did not respond to any of the antiseptic measures recommended by HARRISON (1936) *e.g.* hydrogen peroxide, salvarsan solution, *bis*-hydroxybromophenyl sulphide.   The last three fuadin injections had given rise to intense arthralgia and myalgia so that suspension of the fuadin course was necessary.

On **22.7.39,** the patient was given a course of M. & B. 693, 3·0 grammes daily, for 4 days.   This produced headache, nausea and epigastric pain.   On **27.7.39,** the granulomata and balanitis had completely disappeared, leaving no scarring.   Periodic examinations up to the present time (**18.11.39**) have shown no return of the condition.

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CASE · 4.

O. O., negro labourer, aged 28, first seen on 10th August, 1939, when he complained of an ulcer on the prepuce and a swelling in the right groin.

On Examination.—Well-built, muscular subject.   No lesions found in the cardio-vascular, pulmonary, alimentary or central nervous systems.   In the right groin was a bubo, the size of a walnut, non-fluctuant and unattached to skin or deep structures.   On the inner surface of the prepuce, which was oedematous, was an ulcer, 2 cm. in diameter.

Laboratory Findings.—Scrapings of the ulcer showed Donovan bodies. Frei test positive.

Treatment and Progress.—On **10.8.39**, the patient was given M. & B. 693, 3·0 grammes daily, for 4 days ; the ulcer being treated locally with lotio rubra. On **12.8.39**, no improvement having been noted, patient was given fuadin, 3·5 c.c. Decrease in size of both bubo and ulcer was noted on **15.8.39** when fuadin, 5·0 c.c. was given. Fuadin, 5·0 c.c., was also given on **17.8.39** and **21.8.39** ; on the latter date M. & B. 693, 2·5 grammes daily, for 2 days, was given. The bubo had entirely disappeared and the ulcer was almost healed on **23.8.39**, on which date fuadin, 5 c.c. was given together with M. & B. 693, 2·0 grammes daily for 4 days. On **29.8.39** and **30.8.39**, further injections of fuadin (5 c.c.) were given and on the latter date M. & B. 693, 2·0 grammes daily, for 4 days, was given. On **31.8.39**, the patient complained of pain in the knees, fever and insomnia. Temperature 100·0° F., no malarial parasites in blood. From **1.9.39** to **8.9.39**, the patient was given four injections of fuadin (each 5 c.c.) and on the latter date was discharged fit. Routine re-examinations have revealed no relapse to date (**18.11.39**).

Observations.

This is a mixed infection—lymphopathia venereum and ulcerative granuloma —and, if treated with fuadin alone *might* have shown an antimony-resistance (EARLE, 1938), especially as the penis was oedematous. As has already been shown (EARLE, 1939b) the favourable response of the lymphopathia venereum was due to the action of M. & B. 693.

The response of the lesion (after ten injections of fuadin), together with the fact that no relapse occurred, suggests that the antimony compound was not alone responsible, but that the combination with M. & B. 693 brought about the favourable result. Of the side-effects, the arthralgia was due to the fuadin (WILLIAMSON and co-workers, 1933 ; SÉZARY and BOLGERT, 1935 ; PARDO-CASTELLÓ and co-workers, 1938) and the insomnia to M. & B. 693 (EARLE, 1939c). The pyrexia could not be accounted for.

CASE 5.

R. R., negro, rigman, aged 26, was admitted to the dispensary on 22nd August, 1939, following an accident, in which he had fallen on to a nail, tearing his scrotum. Whilst examining the site of the injury an ulcerative granuloma of the left groin and perineal region was observed. This lesion had been present for over 2 months and the patient had attended a venereal diseases clinic and received a course of injections (? tartar emetic) but these being painful and producing no apparent amelioration he had defaulted and continued to treat himself with patent ointments and herbal remedies. The lesion had continued to spread.

On Examination.—A muscular man. No lesions found in cardio-vascular, pulmonary, alimentary or central nervous systems. An ulcerative granuloma of the dry nodular type extended from the left groin to the perinaeum.

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and that on the right, 1·0 cm. A third granuloma was seen on the right side of the fraenum extending along the prepuce. The lesions were of the ulcus molle type (RAJAM, 1937), and were tender on palpation.

Laboratory Findings.—Wassermann reaction negative. Scrapings from the ulcers showed Donovan bodies but no *Treponema pallidum.*

Treatment and Progress.—Between **30.6.39** and **20.7.39,** the patient was given eleven injections of fuadin (sodium antimony-III-*bis*-catecholdisulphonate of sodium in a 6·3 per cent. aqueous solution) totalling 53 c.c. The left-sided lesion appeared to get smaller, but the other two increased in size and the prepuce became oedematous. No enlargement of the inguinal lymphatics occurred. An erosive balanitis developed on the left side of the fraenum, which did not respond to any of the antiseptic measures recommended by HARRISON (1936) *e.g.* hydrogen peroxide, salvarsan solution, *bis*-hydroxybromophenyl sulphide. The last three fuadin injections had given rise to intense arthralgia and myalgia so that suspension of the fuadin course was necessary.

On **22.7.39,** the patient was given a course of M. & B. 693, 3·0 grammes daily, for 4 days. This produced headache, nausea and epigastric pain. On **27.7.39,** the granulomata and balanitis had completely disappeared, leaving no scarring. Periodic examinations up to the present time (**18.11.39**) have shown no return of the condition.

Observations.

The dramatic termination of this case was undoubtedly due to the M. & B. 693 ; the action of this drug resembling that in the case described by Ross (1939) where cure was obtained in 13 days. It must be remembered, however, that the course of fuadin may have rendered the lesions more amenable to the M. & B. 693.

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Laboratory Findings.—Scrapings of the ulcer showed Donovan bodies. Frei test positive.

Treatment and Progress.—On **10.8.39**, the patient was given M. & B. 693, 3·0 grammes daily, for 4 days ; the ulcer being treated locally with lotio rubra. On **12.8.39**, no improvement having been noted, patient was given fuadin, 3·5 c.c. Decrease in size of both bubo and ulcer was noted on **15.8.39** when fuadin, 5·0 c.c. was given. Fuadin, 5·0 c.c., was also given on **17.8.39** and **21.8.39** ; on the latter date M. & B. 693, 2·5 grammes daily, for 2 days, was given. The bubo had entirely disappeared and the ulcer was almost healed on **23.8.39**, on which date fuadin, 5 c.c. was given together with M. & B. 693, 2·0 grammes daily for 4 days. On **29.8.39** and **30.8.39**, further injections of fuadin (5 c.c.) were given and on the latter date M. & B. 693, 2·0 grammes daily, for 4 days, was given. On **31.8.39**, the patient complained of pain in the knees, fever and insomnia. Temperature 100·0° F., no malarial parasites in blood. From **1.9.39** to **8.9.39**, the patient was given four injections of fuadin (each 5 c.c.) and on the latter date was discharged fit. Routine re-examinations have revealed no relapse to date (**18.11.39**).

Observations.

This is a mixed infection—lymphopathia venereum and ulcerative granuloma —and, if treated with fuadin alone *might* have shown an antimony-resistance (EARLE, 1938), especially as the penis was oedematous. As has already been shown (EARLE, 1939b) the favourable response of the lymphopathia venereum was due to the action of M. & B. 693.

The response of the lesion (after ten injections of fuadin), together with the fact that no relapse occurred, suggests that the antimony compound was not alone responsible, but that the combination with M. & B. 693 brought about the favourable result. Of the side-effects, the arthralgia was due to the fuadin (WILLIAMSON and co-workers, 1933 ; SÉZARY and BOLGERT, 1935 ; PARDO-CASTELLÓ and co-workers, 1938) and the insomnia to M. & B. 693 (EARLE, 1939c). The pyrexia could not be accounted for.

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On Examination.—A muscular man. No lesions found in cardio-vascular, pulmonary, alimentary or central nervous systems. An ulcerative granuloma of the dry nodular type extended from the left groin to the perinaeum.

Laboratory Findings.—Donovan bodies in scrapings.

Treatment and Progress.—On **22.8.39,** patient received a fuadin injection (3·5 c.c.) and M. & B. 693, 3·0 grammes daily for 3 days, was given. Injections of fuadin (5 c.c.) were given on **24.8.39, 25.8.39, 29.8.39, 30.8.39, 1.9.39** and **7.9.39.** M. & B. 693, 3 grammes daily for 3 days, was also given on **25.8.39.** On **7.9.39,** the ulceration had healed completely and subsequent weekly examinations have failed to show a relapse.

Observations.

RAJAM (1937), states that a minimum of three to four courses of fuadin, each numbering twelve to eighteen injections should be given. The rapid and permanent cure of the condition with only seven injections of fuadin, can, I think, only be explained by the fact the action of the drug was supplemented by that of M. & B. 693.

CASE 6.

E. A., male, negro, carpenter, aged 42, was first seen on 24th August, 1939· About 20 years previously he had had inguinal adenitis which had subsided without fistula-formation. There remained, however, slight but permanent swelling of the scrotum, more marked on the left side. He now complained of sores of the scrotum and perinaeum, of about 5 weeks' duration.

On Examination.—A well-built subject. No lesions found in cardio-vascular, pulmonary, alimentary or central nervous systems. On the left side of the scrotum, facing the thigh was an almost circular ulcerating granuloma, 8 cm. in its greatest diameter. It was of the dry nodular type (RAJAM, 1937). In the perinaeum was a similar type of lesion 3 cm. in diameter, extending almost to the anterior margin of the anus.

Laboratory Findings.—Scrapings from granulomatous areas showed Donovan bodies.

Treatment and Progress.—On **24.8.39,** an intramuscular injection of 3·5 c.c. of fuadin was given with M. & B. 693, six 0·5 gramme tablets daily for 3 days. On **25.8.39** and **29.8.39,** further injections of fuadin (5 c.c.) were given and in the latter date a further 3 days' supply of M. & B. 693 (3·0 grammes daily) was given. On **30.8.39,** a further injection of fuadin (5 c.c.) was given and lotio rubra dressings to the affected parts initiated. Healing had advanced centrally 0·5 cm. from the original margin by **1.9.39,** when 5 c.c. of fuadin was again given. M. & B. 693, 3·0 grammes daily, for 3 days, together with fuadin 5 c.c. was again given on **2.9.39.** On **7.9.39,** the scrotal lesion measured 2·5 cm. in diameter whilst the perineal lesion had completely healed : 5 c.c. of fuadin was again given and the patient resumed work on a distant part of the oilfield. Reporting again on **22.9.39,** the patient showed complete healing of the scrotal

lesion to form a puckered but painless and pliable scar. The size of the scrotum was slightly diminished. No trace remained of the perineal lesion. Periodic inspections since that date have failed to reveal any occurrence of the condition. The last inspection was on **16.11.39.**

Observations.

Here a rapid and permanent cure was obtained with eight injections of fuadin, supplemented by M. & B. 693 orally.

DISCUSSION.

Although many antimony compounds have been employed in the treatment of ulcerative granuloma it is now generally acknowledged that the compound of choice is the trivalent aromatic salt, fuadin (WILLIAMSON and co-workers, 1933 ; RAJAM, 1937). But even with this compound, especially where ulcerative granuloma is implanted on oedematous tissue, or where lymphopathia venereum co-exists, antimony resistance may develop (EARLE, 1938) so that gross tissue-destruction finally ensues.

Another disadvantage of fuadin is the large number of injections required to bring about cure and prevent relapse. RAJAM (1937) recommends a minimum of three to four courses, each consisting of twelve to eighteen injections. Aside from the expense of the drug, such a long series of injections (which are not always painless) given to ignorant and undisciplined patients in primitive communities invites a large number of defaulters. Unless, also, favourable results, obvious to the patient, make their appearance after a few injections, the treatment may be abandoned.

A drug which will supplement the action of the antimony and bring about a rapid cure, would obviously be of enormous value in this disease. The idea of supplementing the action of antimony is, of course, not new, since HANSCHELL (1929) has already demonstrated the favourable results obtaining by combining protein shock with antimony injections.

Despite the successful case reported by Ross (1939), I believe that sulphanilamide compounds *alone* have an uncertain action in ulcerative granuloma. MANSON-BAHR (1939) records failure of a sulphanilamide compound in a case of ulcerative granuloma due to " the anaerobic streptococcus of synergetic gangrene described by MELENEY ". In Cases 1 and 2 of the present series, large doses of sulphanilamide, given together by mouth (M. & B. 693) and parenterally (neoprontosil) produced but slight response.

In Case 3, after eleven injections of fuadin, which had produced only very slight healing, the rapid healing (5 days) following administration of 12·0 grammes of M. & B. 693, might, I think, be explained by the fact that here the sulphanilamide was acting on ground already prepared by the action of fuadin.

In Cases 4, 5 and 6 where fuadin and M. & B. 693 were administered simultaneously cure resulted after ten, seven and eight injections of fuadin respectively. No relapse has occurred to date. In these cases the rapidity and permanency of cure can, I think, only be explained by the combined action of both drugs. Even when lymphopathia venereum co-existed and oedema of the penis was present, factors which tend to produce delayed healing and antimony resistance, cure of both conditions occurred after ten injections of fuadin, together with M. & B. 693 orally (Case 4).

It can be said, therefore, that therapy of ulcerative granuloma with combined fuadin and sulphanilamide deserves further trial.

SUMMARY.

1. Six cases of ulcerative granuloma treated with varying combinations of antimony salts with sulphanilamide derivatives are described.

2. Disadvantages and limitations of fuadin and sulphanilamide, used alone, are indicated.

3. Favourable results obtained by combining fuadin injections with M. & B. 693 orally are recorded.

4. A case of mixed infection (ulcerative granuloma and lymphopathia venereum) amenable to fuadin combined with M. & B. 693 is included in the series.

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TRYPANOCIDAL ACTIVITY AND ARSENIC CONTENT OF THE CEREBROSPINAL FLUID OF SLEEPING-SICKNESS PATIENTS, AFTER THE ADMINISTRATION OF TRYPARSAMIDE.

BY

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In two previous papers (Hawking, Hennelly and Quastel, 1937, and Hawking, Hennelly and Wales, 1938) a technique has been described for investigating the penetration of arsenical compounds into the cerebrospinal fluid, and for measuring the trypanocidal activity which they produce therein. The compound is administered intravenously and after a suitable interval of time, cerebrospinal fluid is withdrawn; an estimation is made of the maximum dilution which suffices to kill trypanosomes when they are incubated in it *in vitro* for 24 hours, according to the technique of Yorke and Murgatroyd (1930); by this means an indication of the concentration of active trivalent arsenical is obtained. A simultaneous chemical determination of the arsenic content shows the total amount of the compound which has penetrated into the fluid. Using this technique it was shown that only a fraction (1 to 25 per cent.) of the arsenic found in the cerebrospinal fluid after administration of tryparsamide is present in an actively trypanocidal form. It was suggested that this technique would furnish a useful method for testing out new arsenical compounds intended for the treatment of sleeping sickness or of syphilis of the central nervous system; unless the new compound produced a trypanocidal activity in the cerebrospinal fluid equal or superior to that of tryparsamide, it would not be worth undertaking the great expense and labour of testing it out on a long series of patients with human trypanosomiasis in tropical Africa. Various different compounds were tested, but with one exception (orsanine) none appeared equal to tryparsamide.

The results of these two papers were obtained by observations made upon patients (mostly suffering from general paralysis of the insane) in a mental

No.	Name.	Sex.	Age.	Weight Kg.	Previous history	
					Duration.	Treatment.
1	Matac Mazemba	M.	45	40	1 month	
2	Matilo Zilinga	M.	50	45	1 month	B.4
3	Mishu Lodidudi	M.	40	44	1 month	B.4
4	Fuma Bamé	M.	45	—	1 month	
5	Bamédu Nyameta	M.	42	57	1 month	O.A.T., C. 97, B. 0-124, B.4
6	Fuma Maganga	M.	45	51	1 month	B.4
7	Kalanga Magumbi	F.	45	—	3	B.4, T.11
8	Kasela Ona	M.	31	46	2	B.4
9	Mbuya Meme	M.	36	57	4	B.4
10	Kasoma Mag	F.	31	—	11	B.4, T.11
11	Karalo Matoba	M.	45	56	Treated 11 years previously	
12	Kamana Mandé	F.	45	45	2 months	B.2
13	Ngil Sheje	M.	50	45	1	B.4, T.4
14	Mayombo Bakara	M.	31	30	4 years	B.4, T.7
15	Zagoma Mdingye	M.	30	40	3 months	B.5
16	Madia Kadan	M.	40	50	7	B.4, T.14
17	Namba Kabamba	M.	40	50	4	B.4, T.14
18	Madumyda Kadda	M.	45	45	2	
19	Ngambi	M.	45	45	6	B.5
20	Madadani Kayssa	F.	25	42	11	B.5
21	Masaka Kampanya	F.	35	44	2 months	B.5
22	Mabula Kayaka	M.	45	45	2 months	
23	Hangan Makanga	F.	30	41	2 months	
24	Salim Kashba	M.	32	50	4	B.7, T.13
25	Ndumbagwe Mgwa	F.	32	32	12	B.4, 1 year previously.
26	Mazara Nkandi	M.	35	45	14	B.4, T.30. Relapse
27	Kididio Mada	M.	45	35	10 years	Many treatments and relapses
28	Kaharra Nodi	M.	32	50	4	
29	Buchunganai Kagala	M.	—	50	4	Several
30	Nangoma Kamariza	F.	45	43	12	Many
31	Mivo Mazdo	M.	25	40	4	
32	Maguida	M.	45	55	9	Several
33	Mina Kagwanedo	M.	35	42	12	Two
34	Ngoye Kanangba	F.	25	31	5	Several
35	Palanga Kalinga	M.	12	25	5	Many
36	Kafaka Malingo	M.	12	25	5	Several
37	Masane Kafulai	M.	22	—	10	Many
38	Mivo Mamgwa	M.	45	42	4	Several
39	George Lugunda	M.	25	35	11	Many
40	Nanda Sadiya	F.	30	30	12	Treatment, relapse, treatment
41	Bidada Kaharae	M.	30	30	5	Several treatments and relapses
42	Nakafogwe Mama	F.	32	30	12	Two
43	Maganda Mbambo	M.	50	—	9	Several

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SLEEPING SICKNESS PATIENTS.

Condition at time of observations.			C.S.F.		Response to treatment
Body.	Nervous system.	Tryps.	Cells per mm³	Protein mg. per 100 ml.	
Good	Good	0	5	28	—
Emaciated	,,	0	10	18	Good
Good	,,	0	4	48	,,
Tachycardia. Arthritis	Fair	0	4	28	Fair
Good	Good	0	6	13	Good
,,	,,	0	4	13	,,
,,	,,	0	9	30	,,
,,	,,	0	15	28	,,
,,	,,	0	3	17	,,
,,	,,	0	3	13	,,
,,	,,	0	3	30	,,
Thin. Oedema of legs	Fair	0	17	16	Good
Good	Good	0	—	23	—
,,	,,	0	15	24	—
Pain and slight oedema of legs	Fair	0	280	45	Fair
Fair	Headache	—	450	36	Poor
Good	Good	0	87	45	Vision impaired
,,	,,	0	280	42	Fair
,,	Headache. Pains in legs	0	200	54	,,
Thin. Unable to walk	Stupor. tremors. etc.	—	560	52	—
Oedema of legs	Good	0	34	82	Good
Fair	Headache. Pains in legs	0	435	44	—
Good	Headache. Somnolence	—	640	56	—
Poor. Weak. Pains in head and knees	Apathetic. Sight poor	0	30	82	Fair
Good	Stuporous. choreiform symptoms. etc.	0	120	56	Good but incomplete
Emaciated	Lethargy and tremors	0	110	85	,, ,,
Good	Choreiform movements. Blind	+	154	77	Slight. Died 2 months later
,,	Marked choreiform movements	0	180	73	—
Fair. Weak	Fair	0	65	38	—
,, ,,	Good	0	100	40	—
,,	,,	0	70	44	—
Fair. Cough. Weak	,,	0	12	28	—
Fair. Various pains	,,	0	140	72	—
Emaciated. Weak	Apathetic. Vision impaired	0	80	82	Blindness followed 2nd dose tryparsamide
Good	Good	0	70	85	—
Fair. Pains and weakness	Apathetic	0	85	40	—
Good	Good	0	245	45	—
,,	,,	0	25	45	—
Thin. Weak	Slight dementia; vision impaired	0	40	57	Blindness followed single dose tryparsamide
Good	Fair. headache. vision impaired	0	15	30	—
Fair. Weak	Apathetic. Headache	0	30	47	Poor
Thin. Slight oedema of feet	Vision much impaired	0	100	43	Blindness followed 2nd dose tryparsamide
Fair	Fair. Choreiform movements	0	350	57	—

T indicates 1 dose of tryparsamide (2 grammes).

hospital in Great Britain; in such cases, the meningeal lesions (as shown by the cells and protein of the cerebrospinal fluid) are slight or absent. Therefore. it was determined to repeat these investigations upon cases of human trypanosomiasis showing definite abnormality of the cerebrospinal fluid, in order to discover how far, and in what direction, these pathological conditions would modify the penetration and activation of a typical pentavalent compound such as tryparsamide. It was desired to ascertain how far such tests made in Great Britain upon new compounds were reliable in providing information about the use of the same compounds against sleeping sickness in the tropics. Moreover. it is for these patients with trypanosomiasis that tryparsamide is most widely used. Accordingly, the writer spent the second half of 1938 at Kahama in Tanganyika Territory, East Africa, and carried out the investigations here described.

Methods and Clinical Details.

Kahama is a government station about 200 miles south of Lake Victoria. In the late nineteen-twenties, a considerable epidemic of *rhodesiense* sleeping sickness occurred there and the disease has since continued in a sporadic form. Most of the cases studied in the present series were at a chronic stage, *i.e.,* they had been treated in previous years and had shown clinical improvement, then they had relapsed and had been treated again, and so on, a deterioration occurring with each relapse. The clinical details of these patients are given in Table I, where they are classified into three groups, (*a*) first stage cases, and those apparently cured, with approximately normal cerebrospinal fluids, (*b*) second stage cases, with histories of less than one year, (*c*) second stage cases, with histories of more than one year. Many of these patients had received tryparsamide previously, but in all the cases recorded below, a fortnight had elapsed since the last previous dose. The protein content of the cerebrospinal fluids was determined by the Sicard-Cantouble method, precipitating with trichloracetic acid and heat. The estimation of the trypanocidal activity was commenced within an hour of the withdrawal of the fluid. The remainder of the specimen was sent in a glass-stoppered tube by air-mail to Great Britain (being about eight days in transit) where the arsenic content was determined chemically by Mr. S. DIXON, Public Analyst to Cardiff, as on previous occasions. Previous experiments on dilute solutions of trivalent arsenicals stored for some weeks at room temperature had shown that there was no appreciable loss in the amount of arsenic which could be determined chemically. The technique for estimation of trypanocidal activity was identical with that previously used. except that the serum for the nutrient medium was derived from a sheep (always the same sheep) instead of from rabbits. The trypanosomes were obtained from the same Liverpool strain of *T. rhodesiense* as those referred to in the previous papers.

CONTROLS

Cerebrospinal Fluid from Untreated Patients.

In Table II experiments are shown to test whether the cerebrospinal fluid of sleeping-sickness patients possessed any trypanocidal action upon the strain of trypanosomes used, apart from the administration of tryparsamide. These experiments were made at various times, parallel with observations on patients who had received the drug. Three of these control patients had received Bayer 205 on the previous day; but as (1) chemical tests show that Bayer 205 does not pass from the blood into the cerebrospinal fluid except in extremely small amounts (HAWKING, 1940a); (2) Bayer 205 is not trypanocidal *in vitro* except in concentrations of 0·3 mg. per ml. (HAWKING, 1939); (3) Bayer 205 does not render the blood trypanocidal *in vitro* (HAWKING, 1940b)—it is considered that this administration of Bayer 205 does not affect the results.

The table shows that of the eleven specimens examined, no action was exerted in four cases and some trypanocidal activity was observed in seven; in one of these, the activity was slight and might have been due to the trace of blood present (note the red blood corpuscles). In the other six cases, the action observed was of the type which has been designated for convenience as "false positive." This phenomenon has been considered at length in one of the previous papers (HAWKING, HENNELLY and WALES, 1938). As was there described, trypanocidal action due to natural bodies in the cerebrospinal fluid can usually be distinguished from that due to arsenical compounds by means of the time-relations. The action due to the latter manifests itself slowly, requiring about 12-20 hours for its full development; and so the activity observed after 24 hours exposure is 4-8 times as great as that observed after only 6 hours' exposure. The action due to the former develops rapidly and the activity observed after 24 hours is usually no greater than that after 6 hours. Consequently when the activity observed after 24 hours' exposure is no greater than that at 6 hours, it may be concluded that the reading represents a "false positive," *i.e.,* that death of the trypanosomes has been due to some serological constituent of the cerebrospinal fluid, and not to any arsenical compound which may have been administered; such readings are excluded from all calculations of averages. During work in England in cases of general paresis, this result was observed in 5 out of 35 patients. In the remainder of the experiments here described it was observed in 5 out of 63 specimens. Consideration of Table II shows that there is no parallelism between this tendency to spontaneous trypanocidal activity and the amount of protein present in the fluid. Taking the practical technical point of view, it is concluded from these results that in the cerebrospinal fluid of untreated patients, trypanocidal activity is either absent or capable of being distinguished from that due to arsenical compounds. However, in considering the data recorded below, the natural limitations of this type of technique must be borne in mind. *viz.,* that if trypanosomes survive in a certain dilution of fluid. it is clear that there has not been sufficient concentration of arsenical

to kill them; if trypanosomes, however, fail to survive, their death can be assigned to the arsenical with great probability, but it is difficult to exclude all other possibilities in every case. Hence, although the amounts of *active* arsenic in the specimens below may conceivably have been *less* than the readings in a few cases, they could never have been any *greater*.

Clinically, this slight trypanocidal activity of the cerebrospinal fluid of some untreated patients, is of no importance. The strain of trypanosomes used

TABLE II.

SHOWING THE INVESTIGATION OF THE TRYPANOCIDAL ACTIVITY OF THE CEREBROSPINAL
FLUID OF UNTREATED PATIENTS, CONTROLS.

Patient.	C.S.F.			Minimal trypanocidal concentration of C.S.F. within 24 hours.	Remarks.
	Trypanosomes.	Cells per mm.^{3}	Protein mg. per 100 ml.		
2	0	10	18	No action (in 6 hours)	Bayer 205 1 gramme 48 hours previously
4	0	4 (R.B.C. 80)	28	Slight action	Slight blood contamination
16	0	150	55	(1 : 2) False positive	Tryparsamide 2 grammes 7 days previously
20	+	560	52	No action	Morular cells present. Bayer 205 1 gramme 18 hours previously
25	0	24 (R.B.C. 6)	28	„	Bayer 205 1 gramme 4 times, a few days previously
26	0	110	85	„	Bayer 205 1 gramme 18 hours previously
35	0	40	52	(1 : 2) False positive	
36	0	18	40	(1 : 2) „ „	
41	0	30	47	(1 : 2) „ „	Tryparsamide 12 days previously
42	0	100	49	(1 : 4) „ „	
43	0	350	57	(1 : 4) „ „	

was an old laboratory strain, which was very sensitive to the action of human serum; on freshly isolated strains of *T. rhodesiense* (or *T. gambiense*), which possess considerable serum-resistance, such action would be inappreciable. Perhaps the choroid plexuses, being inflamed, have allowed trypanocidal serological bodies to pass into the fluid from the blood.

Cerebrospinal Fluid of Patients Receiving Tryparsamide.—Specimens of cerebrospinal fluid were obtained from patients who had been treated with

ιryparsamide 3 grammes per 70 kg., injected intravenously, and the results of examination are shown in Table III. There is a large variation between one patient and another in the degree of trypanocidal activity observed, and also in the total arsenic content, and moreover the relation between the two is very irregular. The percentage of arsenic which appears to be present in an actively trypanocidal form ranges from 1 to 30 per cent. at 17 hours after the injection of tryparsamide (usually about 5 per cent.) and from 3 to 20 per cent. at 48 hours after injection. The relation between the amounts of total and of trypanocidally active arsenic in the cerebrospinal fluid and its content of cells and protein is also very irregular. The two or three cases, in which unusually large amounts of both kinds of arsenic were observed, occurred in fluids which contained high concentrations of cells and of protein; but the relation is much too inconstant to possess any statistical significance.
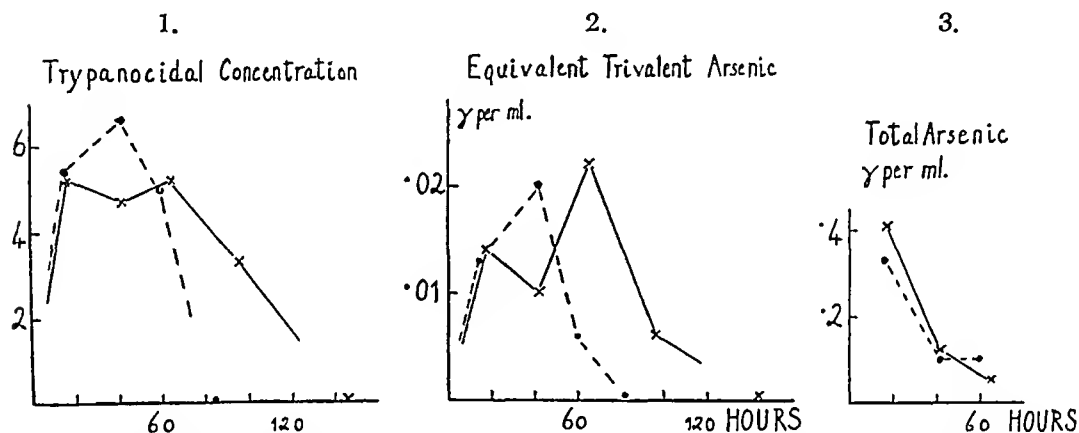
Graph showing (1) the average minimum trypanocidal concentration of cerebro-spinal fluid, (2) the average equivalent concentration of trivalent arsenic, (3) the average amount of total arsenic respectively, following the administration of tryparsamide.
The continuous line refers to the cerebrospinal fluid of patients with trypanosomiasis affecting the cerebral membranes; the dotted line refers to patients with approxi-mately normal cerebral membranes.

The accompanying graphs show the relationship between the time after injection of tryparsamide and the averages of (1) the degree of trypanocidal activity, (2) the equivalent concentration of active arsenic, and (3) the total arsenic content—of the cerebrospinal fluids from patients showing pathological changes therein. The dotted lines in these graphs indicate the similar average values for the fluids of patients without pathological changes, *i.e.*, the cases studied in England (HAWKING, HENNELLY and QUASTEL, 1937) plus the few cases with a normal fluid in the present series.

The differences between these two series of patients are more apparent than real, being mainly due to the inclusion in each group of one or two cases giving

Apparently the inflammation of the choroid plexuses and membranes, due to trypanosomes, does not exert any constant influence on the penetration or activation of pentavalent arsenical compounds, such as tryparsamide. Consequently, comparisons of different arsenic compounds, conducted by this method in temperate countries on patients with approximately normal cerebrospinal fluids, are applicable to the use of the same compounds in the treatment of patients with sleeping-sickness.

Confirmation of this conclusion was sought by the investigation of another arsenical compound, neocryl, which has the formula $NaH.O_2As.C_6H_3.NH.CO.(CH_2)_2.CO.NH.CH_3$. This has been tested on sleeping-sickness patients by MURGATROYD (1937) and ACRES (1937, 1940); the latter reports that the immediate effects of treatment are good but that a high proportion of patients subsequently relapse. A study of this compound made on seven approximately normal patients, which is reported in a previous paper (HAWKING, HENNELLY and QUASTEL, 1937) showed that although its penetration into the cerebrospinal fluid is equal to that of tryparsamide, as judged by the total arsenic content, yet it apparently fails to produce any detectable trypanocidal activity as measured by this technique; presumably it is less readily activated (? reduced to the arsenoxide). In the present work, this compound was given to four sleeping-sickness patients, who had marked pathological changes in the brain membranes as judged by the cells and protein of the cerebrospinal fluid; each received 3 grammes per 70 kg. intravenously and fluid was withdrawn 17 hours later. The average arsenic content of the samples was about 0·3 grammes per ml. showing that the compound had penetrated well; but the trypanocidal activity in all cases was very slight, being less than an equivalent of 0·005γ per ml. of trivalent arsenic. Thus this experimental technique leads to the same conclusions whether it is performed on patients with normal or with pathological cerebrospinal fluids, these conclusions moreover being in agreement with those reached by clinical experience.

Four specimens of cerebrospinal fluid were also examined from patients who had been under treatment for three days with undecane diamidine, either 25 mg. intravenously, or 50 mg. orally, both twice daily (HAWKING, 1940). In two cases the highest concentration of fluid (1:2) killed all the trypanosomes in 24 hours, and in two cases it killed only part of them. A parallel series of tubes containing trypanosomes exposed to known concentrations of the compound suggested that the cerebrospinal fluid content might be about 0·6-0·9γ per ml. This degree of trypanocidal activity is too uncertain and too slight, compared with that of tryparsamide, to possess practical therapeutic value.

Finally, experiments were made to discover whether the degree of trypanocidal activity, recorded above after the administration of tryparsamide, was sufficient to exert any influence upon the local endemic strains of *T. rhodesiense*. Three strains were taken, freshly isolated from patients, Mabula Msali, Maziku Mapalage, and Masalu Manyenye, respectively. The minimum effective dose

of tryparsamide, required to remove trypanosomes from the peripheral blood of rats infected with these various fresh strains or with the old Liverpool strain was 25+, 25, 50, and 25 mg. per 100 grammes, respectively. *In vitro*, the trypanosomes of the three new strains required minimum trypanocidal concentrations (24 hours exposure) of reduced tryparsamide corresponding to about 0·015, 0·007, and 0·01γ per ml. of trivalent arsenic, respectively, thus requiring about 2-8 times the concentration required for the Liverpool strain. When tested *in vitro* against cerebrospinal fluids from patients treated with tryparsamide, the minimum trypanocidal concentrations of the fluids for the different strains were as follows:—

(a) Patients 12 and 13: Liverpool strain, 1:2 trypanocidal; Mabula and and Maziku strains, 1:2 ineffective.

(b) Patient 23: Liverpool strain, 1:4 trypanocidal: Mabula and Maziku strains, 1:2 ineffective.

(c) Patient 21: Liverpool strain, 1:8 trypanocidal: Masalu strain, 1:2 trypanocidal: Mabula strain, 1:2 ineffective.

It is clear that the local strains of *T. rhodesiense* respond much less readily to the tryparsamide, which has penetrated into the cerebrospinal fluid, than the old laboratory strain does.

SUMMARY.

1. In previous papers, a method was described for the investigation of the trypanocidal activity *in vitro*, and of the total arsenic content of human cerebrospinal fluid, after the administration of tryparsamide: these observations were made on patients in a British mental hospital with approximately normal cerebral membranes.

2. In the present work, similar observations were made on patients suffering from *rhodesiense* sleeping-sickness involving the cerebral membranes.

3. The average degree of trypanocidal activity observed in the cerebrospinal fluid after the intravenous injection of tryparsamide (3 grammes per 70 kg.) corresponded to 0·014γ per ml. at 17 hours, 0·01γ per ml. at 42 hours, 0·022γ per ml. at 65 hours, and 0·006γ per ml. at 96 hours; it was inappreciable after 144 hours. The average total arsenic content of the fluid was 0·41γ per ml. after 17 hours, 0·12γ per ml. after 42 hours, and only slight traces after 65 and 96 hours.

4. These quantities are approximately similar to those observed in the series studied in Great Britain; so that conclusions as to the relative merits of different arsenical compounds, reached by this technique in Great Britain, are applicable also when the same compounds are used for the treatment of sleeping sickness in Africa.

5. Apparently the occurrence of trypanosomal lesions of the cerebral membranes, as indicated by the increase of cells and protein in the cerebrospinal

fluid, has no constant influence upon the penetration of tryparsamide into the fluid or on its activation therein.

6. The degree of trypanocidal activity produced by tryparsamide in the fluid of these patients is insufficient to exert much effect upon freshly isolated strains of *T. rhodesiense*.

7. When patients were treated with neocryl or with undecane diamidine, only insignificant degrees of trypanocidal activity were produced in the cerebrospinal fluid.

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FURTHER OBSERVATIONS ON THE TREATMENT OF SLEEPING SICKNESS WITH NEOCRYL.

BY

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The object of this paper is to record the observations made in following-up a number of cases of sleeping sickness treated with neocryl in 1936-37 at the request of the Therapeutic Trials Committee of the Medical Research Council; the immediate results of the treatment have been recorded in a previous paper (ACRES, 1937). Since then it has been possible to examine most of the cases periodically within the last two years and so compare the value of the drug with that of tryponarsyl (the Belgian equivalent of tryparsamide). It will be recalled that dosage with neocryl was calculated on the body weight of the patient and corresponded closely with the dosage used for tryparsamide by CHESTERMAN (1932).

CONTROL OF CASES AND DIAGNOSIS OF RELAPSE.

If, when the treatment with neocryl was completed, gland examination was negative and the cell-count of the cerebrospinal fluid within the limits of normal (five cells or less per c.mm.) then the patient was told to report for further examination after 6 months. If, however, the cell content of the C.S.F. was raised or if there was any doubt about the clinical condition of the patient, then he was told to report within a shorter period of say 2 or 3 months. As was recorded in the addendum of the previous paper, most of the cases were examined before 6 months had elapsed after the courses of treatment, since it had become apparent that there were many relapses.

If on control examination, gland examination was still negative and the cell count of the C.S.F. remained within the limits of normal then a further period of 6 months elapsed before the next control was made. A case which showed no sign of relapse and was in good general condition at least 2 years after treatment was completed was considered cured.

Diagnosis of relapse was made if the trypanosome re-appeared in the blood or gland juice or if, with decline in general condition, a raised cell count in the C.S.F. was recorded. It is perhaps necessary to amplify this latter statement for, as will be found by reference to Table A, at least one case (Case C.4) showed

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*A follow-up report to the Therapeutic Trials Committee of the Medical Research Council.

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TABLE A.

Group	No.	Gland Puncture	C.S.F. Cells	Treatment with Neocryl	Gland Puncture	C.S.F. Cells	Observations	Controls
A	1	+	5	2·0 gm. weekly Total =24 gm.	—	3	Condition good. No symptoms	10 months after treatment Glands =Neg. C.S.F.=3 cells
	2	+	2	2·5 gm. weekly Total =30 gm.	—	2	,, ,,	5 months after treatment Glands =Neg. C.S.F.=2 cells
	3	+	Blood	2 gm. weekly Total =24 gm.	—	6	,, ,,	Approx. 1 year after treatment Glands =Neg. C.S.F.=29 cells
	4	+	7	1·5 gm. weekly Total =18 gm.	—	2	,, ,,	4 months after treatment Glands =Neg. C.S.F.=1 cell
	5	+	2	3·0 gm. weekly Total =36 gm.	—	5	,, ,,	8 months after treatment Glands =Neg. C.S.F.=2 cells
	6	+	15	2·5 gm. weekly Total =30 gm.	—	2	,, ,,	6 months after treatment Glands =Neg. C.S.F.= 4 cells
B	1	+	18	1·5 gm. ×1, then 2 gm. twice weekly Total =27·5 gm.	—	5	,, ,,	6 months after treatment Glands =Neg. C.S.F.=32 cells
	2	+	23	2·0 gm. twice weekly Total =32 gm.	—	5	,, ,,	6 months after treatment Glands =Neg. C.S.F.=23 cells
	3	+	10	2·0 gm. twice weekly Total =30 gm.	—	Blood	,, ,,	5 months after treatment Glands =Neg. C.S.F.=3 cells
C	1	—	420	3·5 gm. weekly Total =42 gm.	—	30	Feels better but reported six weeks after treatment not feeling well	6 weeks after treatment Glands =Neg. C.S.F.=12 cells 24 months after treatment Glands =Neg. C.S.F.=2 cells
	2	—	330	1·5 gm. weekly Total =18 gm.	—	130	Only slightly improved	6 weeks after treatment Onset of "fits" : Sleeping
	3	—	950	3·0 gm. weekly Total =36 gm.	—	16	No longer complains of symptoms	5 months after treatment Glands =Neg. C.S.F.=7 cells
	4	—	253	3·0 gm. weekly Total =36 gm.	—	30	Feels well but control lumbar puncture 6 weeks after = 120 cells	6 months after treatment Glands =Neg. C.S.F.=71 cells 36 months after treatment Glands =Neg. C.S.F.=3 cells Alb. 0·03
	5	+	34	2·0 gm. weekly Total =24 gm.	—	4	Feels well, no symptoms	4 months after treatment Glands =Neg. C.S.F.=22 cells
	6	+	258	2 gm. twice weekly then 3 gm. weekly Total =33 gm.	+	32	Feels well : treated with 2×1 gm. Bayer 205 to sterilize blood	9 months after treatment Glands =Neg. C.S.F.=290 cells
	7	—	247	3·5 gm. weekly Total =42 gm.	—	18	Feels well, no sleeping	3 months after treatment Glands =Neg. C.S.F.=140 cells
	8	+	55 Tryps. +	9×3·5 gm., 3×3·0 gm. Total =40·5 gm.	—	12	Feels and seems well	3 months after treatment Glands =Neg. C.S.F.=92 cells
	9	—	316	2·5 gm. weekly Total =30 gm.	—	38	Feels better but still some headache	4 months after treatment Glands =Neg. C.S.F.=350 cells
	10	+	200	2·0 gm. weekly Total =24 gm.	—	58	Feels and seems much improved	3 months after treatment Glands =Neg. C.S.F.=415 cells
D	1	+	43	3·0 gm. twice weekly Total =39 gm.	—	44	Feels well	6 months after treatment Glands =Neg. C.S.F.= 90 cells
	2	+	150	2×3 gm. weekly then 2 gm. twice weekly Total =30 gm.	—	24	No sleeping during the day : general condition good	4 months after treatment Glands =Neg. C.S.F.=103 cells

TABLE A.

Controls.			Result.
18 months after treatment Glands=Neg. C.S.F.=3 cells	2 years after treatment Glands=Neg. C.S.F.=1 cell		Considered as CURED
22 months after treatment Glands=Neg. C.S.F.=4 cells	2 years and 9 months after treatment Glands=Neg. C.S.F.=2 cells. Alb. <0·02 per cent.		" "
ABSCONDED	2 years after treatment Glands=Neg. Blood Tryps.+. C.S.F. 154 cells	General condition : bad, tremulous —sleeping	RELAPSE ; see Table B
9 months after treatment Glands=Neg. C.S.F.=4 cells	21 months after treatment Glands=Neg. C.S.F.=2 cells	33 months after treatment Glands=Neg. C.S.F.=2 cells Alb. 0·02 per cent.	Considered as CURED
20 months after treatment Glands=Neg. C.S.F.=3 cells	31 months after treatment Glands= Neg. C.S.F.=2 cells. Alb. 0·02 per cent.		" "
11 months after treatment Glands=Neg. C.S.F.=3 cells	18 months after treatment Glands=Neg. C.S.F.=3 cells	24 months after treatment Glands=Neg. C.S.F.=4 cells	" "
General condition poor			RELAPSE ; see Table B
General condition unsatisfactory			" " "
22 months after treatment Glands=Neg.. C.S.F.=3 cells	34 months after treatment Glands=Neg. C.S.F.=2 cells		Considered as CURED
5 months after treatment Glands=Neg. C.S.F.=4 cells 30 months after treatment Glands=Neg. C.S.F.=5 cells	12 months after treatment Glands=Neg. C.S.F.=4 cells 35 months after treatment Glands=Neg. C.S.F.=2 cells	18 months after treatment Glands=Neg. C.S.F.=2 cells	Examinations normal ; residual symptom of " fits " ; ? permanent lesion; considered as CURED
C.S.F.=750 cells (approx.). Trypanosomes +			RELAPSE ; see Table B
21 months after treatment Glands=Neg. C.S.F.=5 cells	33 months after treatment Glands=Neg. C.S.F.=4 cells. Alb. 0·02 per cent.		Considered as CURED
12 months after treatment Glands=Neg. C.S.F.=23 cells	15 months after treatment Glands=Neg. C.S.F.=4 cells	24 months after treatment Glands=Neg. C.S.F.=3 cells	Slow favourable reaction to neocryl ; considered as CURED
			RELAPSE ; see Table B
Bad general condition			" " "
" "			" " "
" "			" " "
" "			" " "
" "			" " "
			" " "

on control examination a pathological fluid, but treatment was not given since the general condition of the patient was so good. Careful observation showed in this case that the cell count became normal without further treatment 2 years afterwards; the significance of this will be discussed later. Our experience has been that the cell count *alone* is not always a satisfactory guide in the control of cases after treatment.

It is realised that it would have been more accurate to have examined the albumin-content of the spinal fluid in the control observations but circumstances made this impossible.

RESULTS OF CONTROLS.

These are shown in two tables as follows:—

> *Table A.*—Showing (1) the controls of cases which were cured by by neocryl; (2) the earlier controls of those cases which relapsed after neocryl treatment.
> *Table B.*—Showing the further treatment and controls of cases which relapsed after neocryl treatment.

SUMMARY OF RESULTS OBTAINED WITH ONE COURSE OF NEOCRYL (TABLE A).

Group A.—First Stage Cases with Weekly Injections.—Of six cases treated, *five* were in good general condition, had negative gland examination, and a normal cell count in the C.S.F. after a period of 2 years (at least) had elapsed since treatment. One case (Case A.3) relapsed.

Group B.—First Stage with Two Injections Weekly.—Of three cases treated one (Case B.3) was regarded as a cure 2 years and 9 months after treatment; while two cases, B.1 and B.2, showed definite signs and symptoms of relapse.

Group C.—Second Stage with Weekly Injections.—Of ten cases treated in this group, only three (Cases C.1, C.3, and C.4) can be considered as cured with neocryl. The other seven showed definite signs and symptoms of relapse.

Group D.—Second Stage Cases with Injections Twice Weekly.—Both of the cases treated in this group relapsed.

(Group E, which included cases which had been previously treated with tryponarsyl before neocryl was used, has been disregarded. All the cases in this group have since died.)

The above results may be shown in tabular form thus:—

	Treated	Cured	Relapse
First Stage	9	6	3
Second Stage	12	3	9

Comparison with Results Obtained with Tryponarsyl.

It is interesting to compare the results recorded above with those obtained during the same period (1936) by treatment with a similar dosage of tryponarsyl.

The results recorded below include only those cases which it has been possible to control for 2 years after treatment.

	Numbers of Cases.				
	Treated.	Cured.	Still under Observation.	Relapse.	Died.
First Stage (0-15 cells in C.S.F.)	77	69 (89·6 per cent.)	1	6 (7·8 per cent.)	1
Second Stage	67	49 (73·1 per cent.)	4	11 (16·4 per cent.)	3

These figures show that the results obtained with neocryl in first stage cases on the whole compare favourably with those with tryponarsyl, but in second stage cases. neocryl treatment compares very unfavourably with that of tryponarsyl.

DISCUSSION OF CASES CONSIDERED AS CURED BY NEOCRYL (TABLE A).

All the cases cured by neocryl showed an uneventful period of control with the exception of the two cases described below.

Case C.1.—It will be recalled that in this case (and in one other, Case C.3) the trypanosome was never actually demonstrated, but as Sicé observes, the diagnosis of sleeping sickness is justified if a lymphocytosis of the cerebrospinal fluid occurs in a patient from a region where that disease is endemic. This observation has incidentally been confirmed in this series of cases, for in two of them (Cases C.2. and C.9) the presence of trypanosomes was not actually confirmed until it was demonstrated in the C.S.F. when the case had relapsed after treatment. This case (Case C.1) was considerably helped by treatment with neocryl. and although laboratory tests show no signs of active disease. the persistence of epileptiform seizures suggests the possibility of a permanent residual lesion.

Case C.4.—This case, it will be recalled. was previously treated in 1932 for sleeping sickness and in 1936 was found to have a pathological cerebrospinal fluid. The cell-content of the C.S.F. rose slightly after treatment with neocryl and then gradually decreased until 2 years after treatment it was normal and remained so for the following year. If one discounts the possibility of a spontaneous cure. it is suggested that the patient's gradual recovery is accounted for by the fact that during the period of control she was suffering from menopausal symptoms which by diminishing her resistance may have delayed her reaction to treatment.

TABLE B.

Number of Case.		Examinations on which relapse was diagnosed (see Table A).	Treatment given on relapse.	Subsequent course and controls of relapse cases.			
Group.	No.						
A	3	Jan , 1939 Glands = Neg. Blood = Tryps. + C.S.F. = 154 cells	2 ×1 gm. Bayer 205 10 ×2·5 gm. Tryponarsyl	April, 1939 Glands = Neg. Blood = Neg. C.S.F. = 2 cells	Nov., 1939 Glands = Neg. C.S.F. = 6 cells Alb. = 0·02%		
B	1	May, 1937 Glands = Neg. C.S.F. = 32 cells	12 ×4 gm. Neocryl	Sept., 1937 Glands = Neg. C.S.F. = 16 cells	Nov., 1937 Glands = Neg. C.S.F. = 73 cells Condition good	Remained under observation. No treatment	Jan., 1938 Glands = Neg. C.S.F. = 98 cells Alb. = 0·03%
B	2	July, 1937 Glands = Neg. C.S.F. = 23 cells	12 ×3 gm. Tryponarsyl	Oct., 1937 Glands = Neg. C.S.F. = 7 cells	Nov., 1938 Glands = Neg. C.S.F. = 3 cells	Nov., 1939 Glands = Neg. C.S.F. = 3 cells Alb. = less than 0·02%	
C	2	Feb., 1937 Glands = Neg. C.S.F. = 750 cells Tryps. +	12 ×1·5 gm. Tryponarsyl	May, 1937 Glands = Neg. C.S.F. = 85 cells	Oct., 1937 Glands = Neg. C.S.F. = 117 cells	TREATMENT with 3 ×2 gm. 9 ×1·5 gm. Tryponarsyl	Dec., 1937 Glands = Neg. C.S.F. = 116 cells
C	5	June, 1937 Glands = Neg. C.S.F. = 22 cells	12 ×2 gm. Tryponarsyl	Aug., 1938 Glands = Neg. C.S.F. = 2 cells Alb. = 0·02%	Nov., 1938 Glands = Neg. C.S.F. = 55 cells(!)	Nov., 1939 Glands = Neg. C.S.F. = 2 cells Alb. 0·02%	
C	6	Nov., 1937 Glands = Neg. C.S.F. = 290 cells	23·5 gm. Tryponarsyl 0·75 gm. Tartar emetic	April, 1938 Glands = Neg. C.S.F. = 44 cells	Sept., 1938 Glands = Neg. C.S.F. = 220 cells Bad condition	Treatment with 20 ×2 gm. Tryponarsyl	Mar., 1939 Glands = Neg. C.S.F. = 78 cells
C	7	May, 1937 Glands = Neg. C.S.F. = 140 cells	12 ×4 gm. Neocryl	Aug., 1937 Glands = Neg. C.S.F. = 24 cells	Jan., 1938 Glands = Neg. C.S.F. = 565 cells RELAPSE	Treatment w th 3 ×3·5 Tryponarsyl 3 ×0·5 gm. Trystibine 6 ×3·0 gm. Tryponarsyl	
C	8	May, 1937 Glands = Neg. C.S.F. = 92 cells	12 ×4 gm. Neocryl	Aug., 1937 Glands = Neg. C.S.F. = 5 cells	Nov., 1937 Glands = Neg. C.S.F. = 170 cells	Treatment with 37 gm. Tryponarsyl	June, 1938 Glands = Neg. C.S.F. = 14 cells
C	9	July, 1937 Glands = Neg. C.S.F. = 350 cells	12 ×2·5 gm. Tryponarsyl	Sept., 1937 Glands = Neg. C.S.F. = 20 cells	Jan., 1938 Glands = Neg. C.S.F. = 197 cells Tryps. + Alb. 0·05%	Treatment with 20·5 gm. Tryponarsyl 1 gm. Trystibine	
C	10	June, 1937 Glands = Neg. C.S.F. = 415 cells	8 ×2·5 gm. Tryponarsyl	Severe and permanent visual complications appeared after 8 injections		Feb., 1938 Glands = Neg. C.S.F. = 7 cells	June, 1939 Glands = small C.S.F. = 5·5 cells
D	1	May, 1937 Glands = Neg. C.S.F. = 90 cells	12 ×4 gm. Neocryl	Aug., 1937 Glands = Neg. C.S.F. = 19 cells	Jan., 1938 Glands = Neg. C.S.F. = 51 cells	April, 1938 Glands = Neg. C.S.F. = 141 cells RELAPSE	Treatment with 2 ×4 gm. and 10 ×3 gm. Tryponarsyl
D	2	June, 1937 Glands = Neg. C.S.F. = 103 cells	4 ×3 gm. 8 ×2·5 gm. Tryponarsyl	Aug., 1937 Glands = Neg. C.S.F. = 11 cells	Dec., 1937 Glands = Neg. C.S.F. = 2 cells	July, 1938 Glands = Neg. C.S.F. = 5 cells	Oct., 1939 Glands = Neg. C.S.F. = 3 cells

TABLE B.

Subsequent course and controls of relapse cases.					Observations.
					Still remaining under observation but apparently a favourable response to treatment with tryponarsyl.
April, 1938 Glands =Neg. C.S.F. =81 cells Alb. 0·04% Condition worse	TREATMENT WITH 1 gm. Bayer 205 21 gm. Tryponarsyl 3·5 gm. Trystibnic	Aug., 1938 Glands =Neg. C.S.F. =8 cells	Nov., 1938 Glands =Neg. C.S.F. =1 cell Alb. 0·02%	Nov., 1939 Glands =Neg. C.S.F. =5 cells Alb. 0·02%	Immediate and favourable response to other treatment after a second course of neocryl had had no effect.
					Favourable response to treatment with tryponarsyl. Considered as CURED.
Nov., 1938 Glands =small C.S.F. =76 cells	Jan., 1939 Glands =small C.S.F. =36 cells Alb. 0·03%	Nov., 1939 Glands =small Blood =Neg. C.S.F. =55 cells Alb. 0·04%			Still under observation, general condition at last control examination was good. Possibly this case is at last showing a response to other treatment.
					Favourable response to treatment with tryponarsyl. Considered as CURED.
May, 1939 Glands =Neg. C.S.F. =350 cells	July, 1939 Glands =Neg. C.S.F. =345 cells	Nov., 1939 Glands =Neg. C.S.F. =201 cells Alb. + +			This patient has visual complications and seems to be drug resistant. Still remaining under observation and periodical courses of Bayer 205.
April, 1938 Glands =Neg. C.S.F. =36 cells Alb. 0·04%	Nov., 1938 Glands =Neg. C.S F. =100 cells	Treated with 12 injections of { 0·5 gm. Bayer 205 0·5 gm. Tryponarsyl mixed			Died August, 1939, apparently drug-resistant.
Oct., 1939 Glands =Neg. C.S.F. =315 cells Alb. 0·085%					Apparently drug-resistant ; under observation and periodical treatment with Bayer 205.
General condition rapidly worse : unsuccessful attempt at treatment with new trypanocide, Ciba 2654 NSN					Patient died, apparently drug-resistant.
					Apparently a favourable response to treatment with tryponarsyl but possibly a smaller dose might have been as successful while preventing the visual complications.
June, 1938 Glands =Neg. C.S.F. =12 cells	Jan., 1939 Glands =Neg. C.S.F. =5 cells Alb. 0·02%	Nov., 1939 Glands =Neg. C.S.F. =2·5 cells Alb. 0·02%			Immediate and favourable response to tryponarsyl treatment after failure with second course of neocryl.
					Good response to treatment with tryponarsyl.

DISCUSSION OF RELAPSED CASES (TABLE B).

Results of Treatment with a Second Course of Neocryl.—Four relapsed cases (Cases B.1, C.7, C.8, and D.1) were treated with second courses of neocryl injections; each of the patients was a well developed man and in each case twelve weekly injections of 4 grammes of neocryl were given. No toxic effects were recorded and in none of the four cases was there any improvement clinically; eventually all had to be treated with tryponarsyl, to which two (Cases B.1 and D.1) showed a favourable response, while the others have proved resistant. It is interesting to note that one of the patients who benefited by tryponarsyl volunteered the statement that he felt better after treatment with tryponarsyl than he ever did after either of the courses of neocryl.

Results of Treatment with a Course of Tryponarsyl after Relapse after Neocryl.—Eight of the cases (Cases A.3, B.2, C.2, C.5, C.6, C.9, C.10, and D.2), which relapsed after neocryl treatment were treated with a course of tryponarsyl and five of them (Cases A.3, B.2, C.5, C.10 and D.2) responded well. One of the others, Case C.2, seems at long last to be responding to repeated tryponarsyl treatment, the patient being in good general condition and still under observation because of a markedly abnormal C.S.F. Of the remaining Cases C.6 and C.9, the former has been unresponsive to all treatment, while the latter became rapidly worse and died in spite of all treatment given.

The favourable reaction to tryponarsyl which was seen in some cases when treatment with neocryl had not been successful, might be thought to be due to insufficient doses of neocryl being given in the first place, but as shown above it seems quite definite that a second course of neocryl does not cure a relapsed case. It seems legitimate therefore to conclude that neocryl is not so potent a trypanocide as tryponarsyl once the infection has spread to the central nervous system.

SUMMARY AND CONCLUSIONS.

1. Twenty-one cases of sleeping sickness which were treated with neocryl in 1936-37 have been observed over a period of at least two years.

2. The results obtained in first-stage cases compare favourably with those of tryponarsyl.

3. The results obtained in second-stage cases are very disappointing, only three of the twelve cases being cured. This compares very unfavourably with cases treated in the same district and in the same year with tryponarsyl.

4. A second full course of neocryl treatment was given in four relapsed cases without result. Two of the cases became unresponsive to all later treatment with tryponarsyl.

5. Of eight relapsed cases treated later with tryponarsyl six reacted favourably and two were unresponsive.

6. The failure of neocryl in so many of the second stage cases seems to confirm clinically the observation made by HAWKING, HENNELLY and QUASTEL (1937) that the trypanocidal activity of cerebrospinal fluid is less in the case of neocryl than of tryparsamide.

In conclusion I would like to correct a slight error in the previous paper to which my attention has been drawn. The Crylarsan brand of neocryl was supplied through the Association of British Chemical Manufacturers on behalf of Boots Pure Drug Co., Ltd., the British Drug Houses Ltd., Burroughs Wellcome & Co., and May & Baker, Ltd.

My thanks are due to the Government of Congo Belge who, by my appointment as medical officer supervising the region of Bolobo, made it possible for me to follow up successfully these cases treated with neocryl.

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CRAZY PAVEMENT SKIN ERUPTION.

BY

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There is a tendency at the present time to attribute certain types of skin changes in the malnourished to pellagra, or to refer to them as pellagroid. An example of this occurs in the January issue of these TRANSACTIONS, under the title " Infantile Pellagra." In this paper H. C. TROWELL (1940) reviews a number of publications in which certain types of malnutrition in children have been described from many parts of the world. Apparently the commonest sign in these types is oedema; but many, if not all, of the children show a skin eruption, which being irregularly fissured was first described by WILLIAMS (1933) as resembling crazy pavement. Some of these publications state that other signs, such as xerophthalmia, stomatitis, polyneuritis and diarrhoea also occur in some of these children. But the diagnosis of " infantile pellagra " appears to be founded mainly on the crazy pavement eruption.

The nomenclature of malnutrition is a difficult matter, because unlike the infections, there is seldom a single etiological factor, a deficient diet almost always being deficient in more than one factor.

Oedema is not a characteristic of pellagra, and when it is present in the malnourished it is mainly due to protein deficiency, and it is appropriate to refer to it as nutritional oedema.

Skin changes, which may be likened to crazy pavement, are far from uncommon among patients in the wards of the general hospitals in the tropics. The following are two examples :—

Case I.

The patient, age 35, was a fairly well-to-do trader. He had suffered from chronic nephritis for about 2 years. He had had oedema of the legs, and this had subsided on a high protein diet. The skin over the anterior surfaces of the legs was somewhat rough, and was finely and irregularly fissured (*vide* Fig. 1). He stated that his skin had always been somewhat rough, but only recently had been fissured. The face, back of the hands, upper surfaces of the ankles, and the rest of the body was free from these changes. His description of his diets before and after his illness started indicated that they had always been adequate, and had included ample of the protective foodstuffs, such as milk and eggs.

Case II.

The patient, age 30, was of the labouring classes, and was suffering from phthisis. He had patches of superficial epithelium which were darker than the normal skin and were separated by fissures (*vide* Fig. 2). The skin changes occurred only on the legs below the knee ; the upper surfaces of the ankles were not affected. He had lived on the usual curry and rice diets before entering hospital 6 weeks previously, and his descriptions of his diets did not indicate any marked deficiencies.

It may be that in such cases as these there has been deficient absorption or faulty metabolic utilization of certain vitamins, but in the present state of our knowledge we are not justified in making assumptions of this kind, still less to diagnose all such cases as pellagra mainly on the existence of a crazy pavement eruption.

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FIG. I.   FIG. II.

CRAZY PAVEMENT SKIN ERUPTION.

EXPERIMENTAL VISCERAL LEISHMANIASIS IN MAN.

BY

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In a previous paper* it was shown that the transmission of visceral leishmaniasis to human beings debilitated by malignant disease is not easy even when relatively enormous amounts of living cultures of leishmania from the organs of infected Syrian hamsters are repeatedly injected. Out of five cases thus treated only one became infected and this case showed that leishmania stimulate infected cells to phagocytosis, a fact which we have frequently observed in dogs infected with *Leishmania infantum.*

It was suggested that the rapid lytic action of normal human serum on leishmania may protect a human being by destroying the inoculated parasites before they have established themselves in reticulum cells outside the circulation. As previously pointed out the lytic property of human serum cannot be the only factor involved in resistance to leishmania; the serum of the non-susceptible rabbit is far less lytic than that of the susceptible human being.

Experiments with the objects and on the lines previously indicated were continued, and a case of malignant disease was subjected to inoculations of leishmania from spleens of Syrian hamsters infected with a Haifa strain of *L. infantum.* The whole spleen was divided into small fragments and ground up in saline and the total of the resulting mixture inoculated.

HISTORY OF CASE.

10.1.38. A piece of right mamma of a female aet. 42 was excised and a diagnosis of adeno-carcinoma was established by histological examination.

25.1.38. Radical amputation of breast: the glands in the right axilla were found to be involved.

In December, 1939, the patient complained of pain in the left arm and metastases were noted in the supraclavicular glands on both sides, in the left axilla and in the mediastinum. She came under care of Prof. L. HALBERSTADTER of the Cancer Department of Hadassah Rothschild University Hospital.

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*ADLER, S. (1940). Attempts to transmit visceral leishmaniasis to man. Remarks on the histopathology of leishmaniasis. *Trans. R. Soc. trop. Med. Hyg.,* 33 (4), 419.

INOCULATIONS OF *L. infantum.*

Serum from the patient who was jaundiced produced no lysis of flagellates of *L. infantum in vitro.*

9.1.40. Leishmania from spleen of an infected Syrian hamster injected intragluteally.

26.1.40. Ditto.

8.2.40. Leishmania from spleen of a Syrian hamster inoculated into enlarged supraclavicular glands on left side.

25.2.40. Patient died.

POSTMORTEM EXAMINATION.

Dr. H. UNGER of the Department of Pathology of the Hadassah Rothschild University Hospital performed the postmortem examination and kindly provided us with material.

Metastases were found in the supraclavicular glands on both sides, in the left axilla, in the mediastinum (both sides of the aorta) and in the liver which was atrophic, on the pericardium and right pleura.

A few leishmania were found in spleen smears only. Other organs including the glands which had been directly inoculated were negative on microscopic examination.

Histologically nothing of interest from the point of view of kala-azar was found.

The following points should be emphasised : —

(1) The patient aet. 44 was infected experimentally with *L. infantum,* which is relatively rare in adults.

(2) The number of injections and the amount of material injected was considerably less than in some previous cases inoculated with a negative result with *L. donovani,* which is relatively common in adults.

(3) The patient was jaundiced and her serum when examined was not lytic for flagellates of *L. infantum in vitro.*

(4) Parasites were found in the spleen 47 days after the first inoculation. They were not found in the enlarged supraclavicular glands which had been directly inoculated.

Although we cannot draw definite conclusions from a single experiment, nevertheless the relative ease with which the above case was infected suggests that the jaundice, and the accompanying disappearance of the lytic action of serum on leishmania, enabled the parasites to reach the spleen. where they established themselves.

CORRESPONDENCE.

THE FIRST RECORD OF SLEEPING SICKNESS.

To the Editor of the TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

Dr. H. HAROLD SCOTT (1939) in his *History of Tropical Medicine* states that attention has recently been directed by H.R.H. PRINCE OMAR TUSSIM (1931) to a description of sleeping sickness by AL-QUALQUASANDI, an Arab writer of the fourteenth century. The case referred to is that of Mari Jaza, a Sultan of the Malli kingdom, whose condition is described as follows :—

" His end was to be overtaken by the sleeping sickness ('illat an-nawm) which is a disease that frequently befalls the inhabitants of those countries especially their chieftains. Sleep overtakes one of them in such a manner that it is hardly possible to awake him. He (the King) remained in this condition during two years, until he died in the year 775 A.H. (A.D. 1373-1374)."

Attention has been directed to this record also by KRENKOW (1928). The record is of considerable interest not only because it antedates by over 300 years ATKINS's (1734) description of sleeping sickness, but also because another record apparently referring to the same case has found its way into medical literature from an entirely different source. According to CHALMERS and O'FARRELL (1914), " The earliest recorded case of sleeping sickness is the death from lethargy of King Mansa Djata in 1373-74 ; at the time, it is stated, the disease was very common in his country which is situated in the bend of the Niger." These writers cite as their authority SLANE's translation of IBN KHALDOUN's History of the Berbers. The passage in the French original (translated from the Arabic) is given in the *Bulletin of the Sleeping Sickness Bureau* (1910) as follows :—

" Il fut enfin atteint de léthargie, maladie très commune dans ce pays et qui attaque surtout les gens haut placés. Cette indisposition commence par des accés periodiques et réduit, enfin, le malade à un tel état qu'à peine peut-on le tenir un instant éveillé. Alors, elle se déclare d'une manière permanente, et fait mourir sa victime. Pendant deux années Djata eut à en subir les attaques, et il y succomba l'an 775 (1373-4)."

The Malli Empire (or Mellestine) arose in the thirteenth century, and was the first of the great black Mohammedan kingdoms of the western Sudan. The names Mari and Mansa may be regarded as synonymous, both being titles having the significance of " King." There can be little doubt that the two records refer to the same individual, and indicate that sleeping sickness was probably recognized in the region of the Niger as early as the fourteenth century. Unfortunately our knowledge of the Malli Empire is at present very scanty, being practically limited to fragments scattered through the works of a few medieval Arab writers, of whom IBN BATUTA (1303-1377) was the only one to visit the Sudan. He does not mention sleeping sickness. IBN KHALDOUN (Abu Zeid ibn Mohammed ibn Mohammed ibn Khaldoun) the great historian of the Berbers, and one of the most illustrious Arab scholars of the fourteenth century, did not personally visit the Sudan ; his information about Mari Jata was derived from a native of Sijilmasa who had lived in Malli.

IBN KHALDOUN died in Cairo in 1406 A.D. AL-QUALQUASANDI died 12 years later, in 1418, and it is possible that his description of the disease was taken from that of IBN KHALDOUN.

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COMMUNICATIONS.*

THE MORPHOLOGY OF MALARIAL PARASITES IN THICK BLOOD FILMS.

PART III.

PLASMODIUM MALARIAE.

BY

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AND

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This paper, the third of a series (FIELD and LE FLEMING, 1939, 1940) on the morphology of malarial parasites in thick blood films, attempts to describe the thick film appearances of *Plasmodium malariae*.

Technical details of the methods of staining the films from which the drawings were prepared and on which the descriptions are based were recorded in the first paper of the series (FIELD and LE FLEMING, 1939). Colour drawings were made direct from the microscope; they attempt to reproduce what was seen without diagrammatic simplification. Photomicrographs were taken from films

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* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

which had been stained rather more intensely than is necessary for visual examination—a slight modification of the staining procedure originally described which gave better photographic contrast.*

Early Trophozoites.

The familiar appearance of young trophozoites in fixed thin films is modified in stained thick films only in their size and in the arrangement of the cytoplasm. The smooth " ring " contour is usually lost: an incomplete ring formation may persist but more often the vacuole of the parasite is collapsed and the cytoplasm is contracted to a curved blue wisp terminating in a relatively large bead of chromatin. The common patterns are those which *P. falciparum* is liable to assume in thick blood films—" commas," " exclamation-marks," " question-marks," as they may be termed for brevity of description—and indeed at at this stage the two parasites bear a close resemblance. Like most malarial parasites in thick blood films they tend to appear smaller than fixed parasites at a corresponding stage of growth in thin films (Plate XI, Fig. 1, 2).

The nuclear chromatin is a single bead, large in relation to the amount of cytoplasm. Doubled chromatin is rare. The " bird's-eye " arrangement of the chromatin and cytoplasm which is a common appearance in fixed thin films is not seen. Chromatin and cytoplasm are usually in fairly close association but may occasionally be separated by a considerable gap.

All trace of the host cell is lost. Ziemann's stippling is never seen. Pigment is absent.

This stage lasts but a few hours and in the general run of routine blood examinations young unpigmented trophozoites are comparatively uncommon. They are, moreover, seldom seen alone but tend to be associated with a few ripe segmenting forms.

Late Trophozoites.

The " comma " state of the young trophozoite is of short duration. The cytoplasm contracts and envelops the chromatin with little or no tendency to dispersion and the parasites become compact and rounded—a form which is retained until schizogony begins. At first they are small but in the course of 24 to 36 hours they grow from 3μ or less to 6μ or more in diameter. Throughout this period they tend to retain a solid-looking spherical or ovoid form. There is little or no tendency to the breaking up and scattering of the cytoplasm which is a characteristic feature of *P. vivax* at this stage : at all stages, and particularly at this stage, *P. malariae* seems to offer considerable resistance to

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* The thick blood films from which the photomicrographs were made were dried for about 12 hours, "fixed" by immersion for 1 second in a 0·5 per cent. solution of methylene blue, rinsed for few seconds in tap water to remove the excess stain and finally stained for 2 hours in a solution containing 20 drops of Giemsa stain to 100 c.c. of water buffered to a. pH of 7·2 with phosphates.

the destructive effects of the thick film staining process and to contract rather than scatter. The linear forms which are often seen in thin films as equatorial bands stretched across the host cells are, for example, usually contracted in thick films to spheres or ovoids and seldom if ever appear as " bands."

The chromatin is a single bead ; it is no longer isolated from the cytoplasm as in the earlier states of growth but is enveloped and often obscured.

Pigment appears early. Even in the small compact forms but a few hours removed from the early " comma " stage small granules of pigment may be seen scattered through the cytoplasm. Later the pigment may be so profuse that the whole parasite has a yellowish tinge. In faded or understained films the pigment may be so prominent that the dominant colour of the parasite is yellowish rather than blue. This early and marked formation of pigment is more obvious in thick films than in fixed thin films and is of importance in species diagnosis.

Typically the parasite has three distinctive features at this stage : compact texture, round or ovoid contour and early and profuse pigment formation.

Early Schizonts.

The dense texture of the trophozoite is retained through early schizogony. The parasite is still roughly round or oval in shape, compact and with little tendency to the thinning and fraying of outline that is characteristic of *P. vivax* at this and earlier stages. The general ground of the parasite is the blue-stained cytoplasm which is locally or diffusely coloured yellow-green from the dense charge of pigment.

The chromatin is divided into several irregular masses sometimes clearly defined, sometimes visible merely as a vague purplish condensation within the cytoplasm or sometimes even completely obscured.

The pigment is characteristic ; diffuse, and often so abundant that the internal detail of the parasite is seen as through a yellow-green haze. It appears as small granules varying in colour from yellow to green depending on the depth of the cytoplasm through which it is seen. At the parasite fringe pigment is often seen isolated.

The typical appearance during early schizogony is that of a roughly round or oval parasite, compact and heavily pigmented, with a few masses of purple chromatin visible through the cytoplasm and pigment which envelop them.

It may be noted that schizogony tends to occur rather late in growth and the parasite is often approaching full size before visual evidence of segmentation appears.

Advanced Schizonts.

The parasite remains relatively compact but is now less rounded and regular but often scalloped from the projection of developing segments. The cytoplasm is not clearly segmented but still envelops the chromatin segments.

The parasite is still, as it were, a single unit.

The chromatin is divided into from five to ten segments, usually eight, irregularly arranged throughout the parasite. It appears purple in colour and is usually well defined but sometimes is merged into the cytoplasm so that the outlines are not clear. Individual rounded masses often project beyond the outline of the parasite. Occasionally one or more merozoites separate prematurely from the parasite and are seen lying alongside.

The pigment is often still diffuse and scattered between the chromatin masses and around the periphery : or there may be a tendency to concentration towards one side so that the parasite appears to have a blue zone with purple condensations of chromatin shading into a yellow zone of pigment. We may note, in passing, that pigment tends to concentrate late and occasionally may still be diffuse even in fully mature schizonts—a useful point of distinction from *P. falciparum* in which the concentration of pigment is early and intense.

The typical late schizont is a fairly compact parasite with an irregularly scalloped contour, with undivided blue cytoplasm merging into discrete segments of chromatin and with profuse granules of pigment in one or two loose collections, or scattered between segments and around the periphery.

Mature Schizonts.

The typical mature schizont is a compact collection of pigment granules around which are scattered from six to ten, but fairly consistently eight, merozoites. The merozoites are small ovoid bodies with a centre of reddish purple chromatin and a slight loose covering of blue cytoplasm. They appear to consist predominantly of nuclear material ; often, in fact, the cytoplasm has disappeared and the merozoite cluster is a collection of minute vividly-stained sharply-defined ovoids of chromatin.

The pigment is usually concentrated and individual granules are not as a rule visible but coalescence is never so dense and homogeneous as with *P. falciparum.* Occasionally the pigment is loosely scattered throughout the merozoite cluster.

Fully differentiated merozoites are usually clearly separated and somewhat dispersed. They have less tendency than have those of *P. vivax* and *P. falciparum* to remain as a close-knit cluster.

Gametocytes.

The gametocytes of *P. malariae* are not as a rule difficult to recognise in thick blood films, though there is considerable morphological variation. They are small, compact, round or oval parasites with abundant pigment and undivided chromatin. They resist the disruptive effects of the thick film process fairly well and often preserve the general form and outline seen in fixed thin films.

The cytoplasm stains blue; the depth of staining varies from dark to very light blue—differences which seem to be related as much to the physical effect of lysis as to any other single factor. Occasionally the cytoplasm cannot be seen and the parasite appears as a single mass of chromatin enveloped in a zone of pigment.

Pigment is profuse : with bright illumination it is often the most obvious feature of the parasite. The granules are not concentrated, as they usually are in the mature schizont, but are arranged as a peripheral fringe encircling the parasite or else scattered irregularly throughout the cytoplasm. The colour of the pigment appears yellow or yellow-green depending on the depth of staining of the blue cytoplasm in which it lies. The granules are somewhat, though not markedly, coarser than those of *P. vivax* at a similar phase of development.

The chromatin may be well defined as a single oval purple-staining mass with no characteristic position within the parasite, or paler, redder and more diffuse. Occasionally the chromatin cannot be seen at all ; the nature of the parasite is then indicated by its size, the dispersion of the pigment and the absence of evidence of segmentation.

The typical gametocyte in the Romanowsky-stained thick film is a fairly compact rounded or oval parasite, small and heavily pigmented, with a single ovoid mass of chromatin, usually deeply stained and clearly defined but sometimes pale, diffuse and vague.

Young gametocytes may be difficult to differentiate from advanced trophozoites as chromatin division in the asexual forms is often delayed until the parasite is approaching full size. Their identity is suggested by the absence of chromatin division at the stage of growth when schizogony would be expected, but identification with certainty may be impossible.

The differentiation of sex is difficult. Some gametocytes have compact well-defined chromatin and are presumably females ; others have pale diffuse chromatin and may be males. No constant relation between the appearance of the chromatin and the colour and depth of staining of the cytoplasm or the coarseness or arrangement of the pigment has been noted. The determination of sex in thick films is hence believed to be unreliable. Exflagellation of the male gametocyte in thick films, prepared and stained by the method described in the first paper of this series, has not been seen.

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———— ————. (1940). Part II. *Plasmodium falciparum*. *Ibid.*, 33 (5), 507.

DESCRIPTION OF TEXT FIGURE.

(*a*) Young trophozoites seldom retain the " ring " form seen in fixed thin films ; the " comma " appearance shown in these tracings is more usual.

This early phase of growth at which the chromatin bead is not yet enveloped by the cytoplasm lasts but a few hours and hence, in the general run of routine blood examinations, early trophozoites are much less often seen than the more advanced forms. When present they are usually associated with older compact forms or with mature schizonts—a fact which aids distinction from *P. falciparum* trophozoites which they closely resemble.

(*b*) Envelopment of the chromatin bead by the cytoplasm, contraction to a compact rounded form and the appearance of pigment, occur very early in growth.

(*b*), (*c*) and (*d*). The parasite tends to remain rounded and compact from the time when the chromatin is first enveloped by the cytoplasm and pigment appears, until schizogony is clearly evident—a period of 36 hours or more, *i.e.*, for at least half of the schizogony cycle. For this reason these are the forms of *P. malariae* most often seen in routine blood examinations. A close-knit texture, rounded form and abundance of pigment are the main features of *P. malariae* at this period of growth.

(*d*) Visual evidence of chromatin division is often delayed until the parasite is approaching full size. The chromatin is ill-defined and the earliest evidence of division difficult to see. These forms resemble young gametocytes from which, when present only in small numbers, they cannot be distinguished with certainty.

(*e*) Early schizonts are less regular in outline but, even so, there is little tendency to the cytoplasmic dispersion which characterises *P. vivax* in thick blood films. The chromatin is more vividly stained and as a rule is well defined. The granules of pigment are beginning to concentrate into one or two areas.

(*f*) and (*g*). Mature schizonts consist typically of eight merozoites in a loose cluster, and a collection of pigment granules. The ovoids of chromatin in the merozoites are deeply stained. Sometimes, as in (*f*) the chromatin is still invested with a cytoplasmic covering, more often, as in (*g*), the cytoplasm has disappeared. The granules of pigment are usually concentrated but seldom do they appear fused to a homogeneous mass as with *P. falciparum*, and occasionally they may even remain dispersed. The merozoites are often widely scattered (*g*)—a tendency which is more marked with *P. malariae* than with other species.

(*h*) Undivided chromatin and scattered pigment associated with full size characterize the gametocyte. The cytoplasm is often well preserved but may be eroded or even absent. The chromatin may be clearly seen or diffuse and vague—possibly a difference of sex. The pigment is coarse and scattered, often with a tendency to distribution as a peripheral fringe.

These forms closely resemble the mature *P. falciparum* gametocytes which have " rounded off " from commencing maturation during the drying of the film. Identification is aided by the differences in the character of the pigment—the pigment of *P. falciparum* gametocytes tends to appear as coarse rodlets ; that of *P. malariae* as less coarse granules.
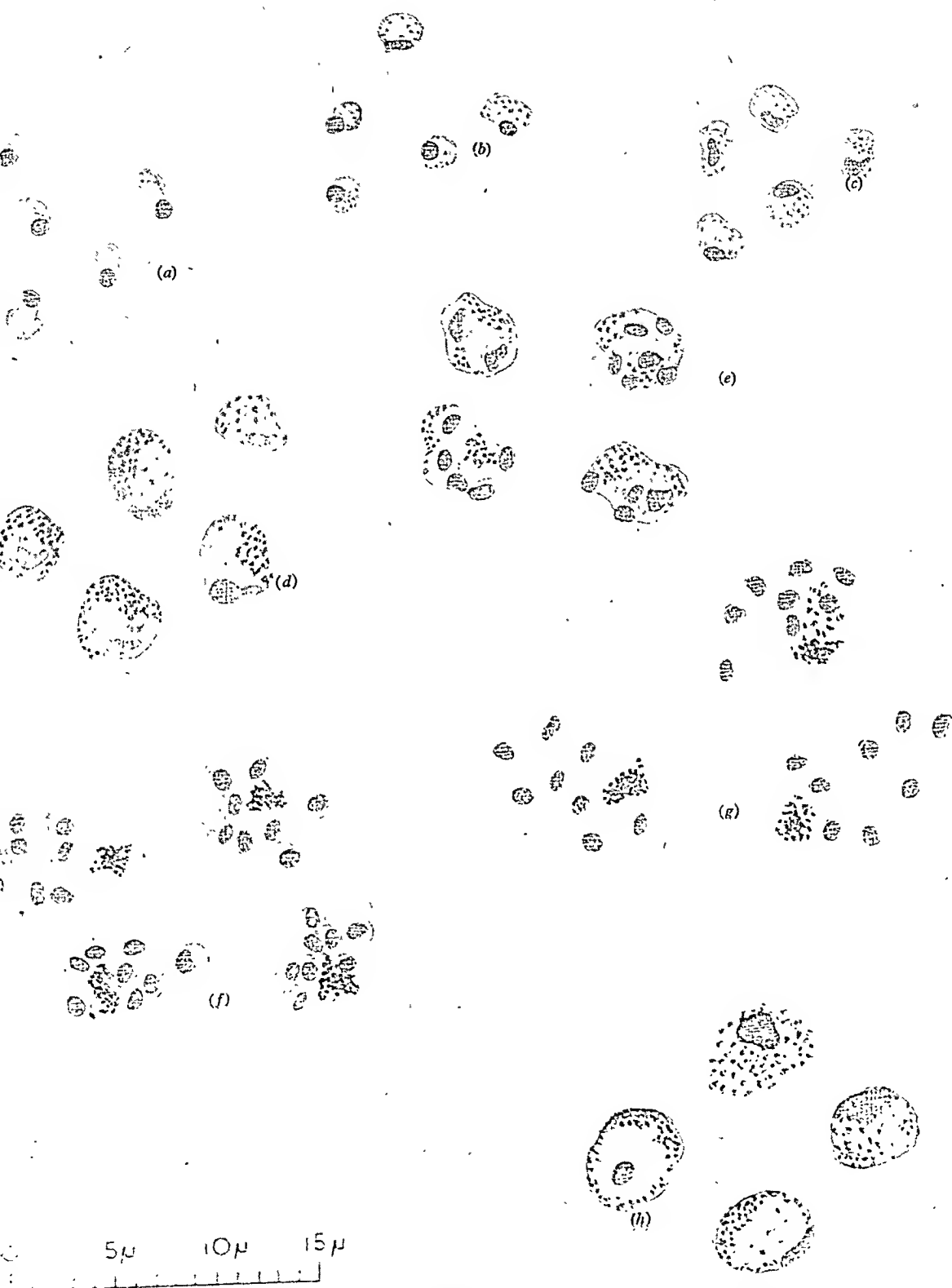
P. malariæ IN GIEMSA-STAINED THICK BLOOD FILMS (tracings from photographs).

PLATE IX.

P. malariae IN GIEMSA-STAINED THIN FILMS.

Uninfected cell.
  1.

Trophozoites.
  2 :   Bird's eye form.
  3 :   Young ovoid form with early pigment formation.
  4 :   Young " band " form.
  5, 6 :   Larger " ovoid " forms.

Schizouts.
  24 :   Early schizont with " band " formation.
  25 :   Early ovoid schizont with persisting vacuolation.
  26 :   An irregular formation fairly common in early schizogony.
  27, 28 :   Advanced schizonts.
  29 :   Mature schizont.

Gametocytes.
  40 to 45.

P. malariae IN GIEMSA-STAINED THICK FILMS.

Trophozoites.
  7, 8, 9 :   Young unpigmented forms with chromatin dot still isolated.
  10 to 23 :   Older trophozoites showing early formation of pigment and
               tendency to early assumption of compact rounded form.

Schizonts.
  30 to 33 :   Chromatin division just beginning.
  34, 35 :   Early schizonts.
  36, 37 :   Advanced schizonts.
  38 :   Mature schizont; this retention by the merozoites of their
               covering of cytoplasm is unusual.
  39 :   Mature schizont; the usual appearance of merozoites as
               ovoids of chromatin divested of cytoplasm. The pigment
               in this mature schizont is shown as concentrated; some-
               times it is scattered as granules among the merozoites.

Gametocytes.
  46, 47, 49 :   Some degree of erosion of the cytoplasm as in these examples,
                     is not uncommon and chromatin may be poorly defined.
                     Differentiation from eroded advanced trophozoites is then
                     difficult.
  48, 50, 51, 52, 53 :   Typical thick film appearance.
  51, 52 :   Dispersion to a size larger than in fixed thin films sometimes
                     occurs.
  54 :   Occasionally the cytoplasm has disappeared and there remains
                     only a prominent blob of chromatin with a halo of pigment
                     granules.

1   2   3   4   5   6

7   9   12   13   18   20   21

8   10   11   14   15   16   17   19   22   23

24   25   26   27   28   29

30   32   34   36   38   39

31   33   35   37   38

40   41   42   43   44   45

46   48   50   52   53

47   51

49   54

HE

O   5 μ   10μ   15μ
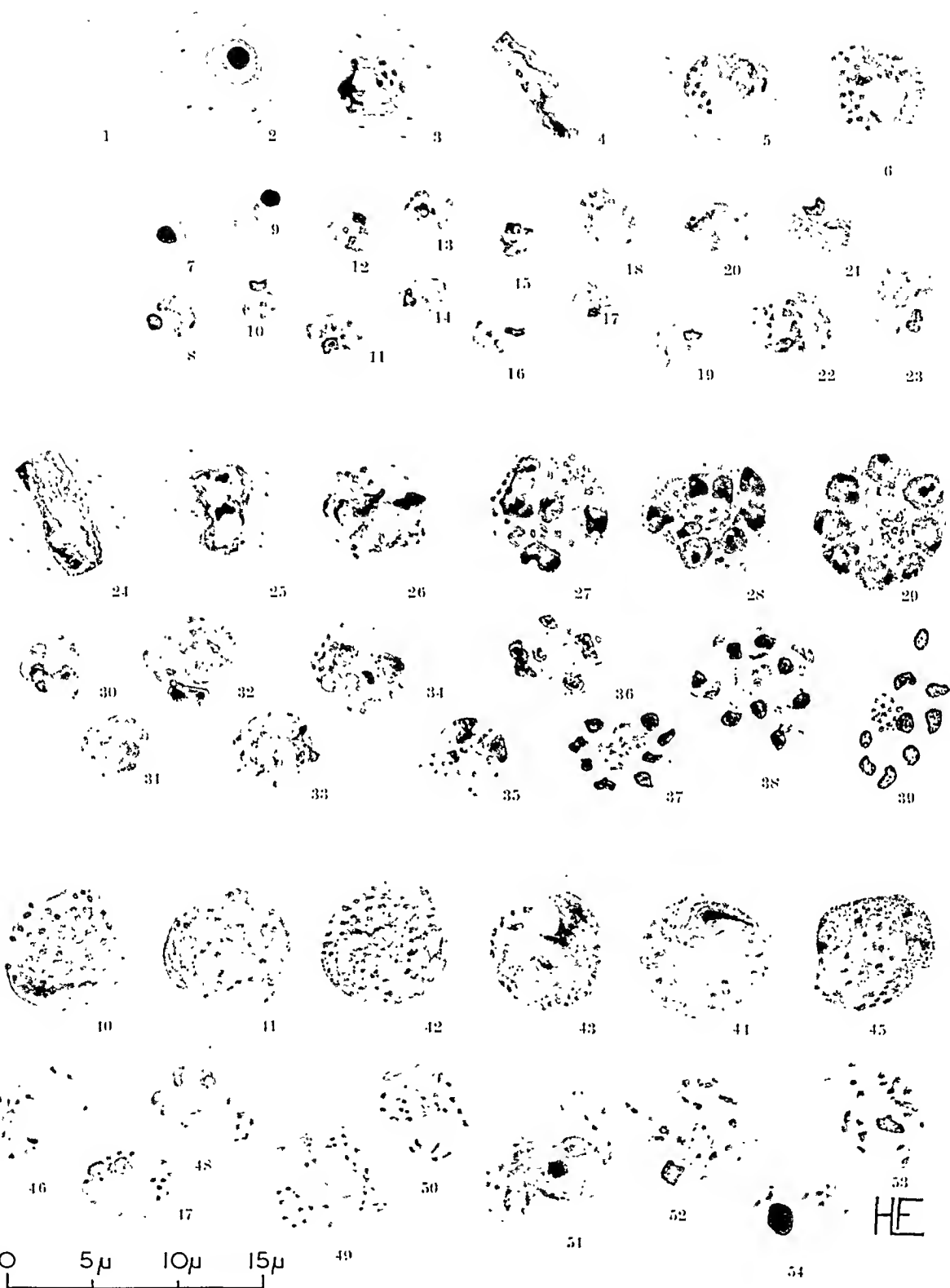
PLATE IX.

PLATE X.

P. malariae IN GIEMSA-STAINED THIN AND THICK BLOOD FILMS.

The two half-fields illustrate characteristic differences in the thick and thin film appearances of *P. malariae* at the trophozoite stage of development.

Left half-field—thin film :—

        " Band " trophozoite.
        " Ovoid " trophozoite.
        Gametocyte.

Right half-field—thick film :—

        Young unpigmented trophozoites at the " comma " stage with chromatin still isolated.
        Slightly older trophozoites with early pigment formation.
        Advanced trophozoites.
        Gametocytes.
        Also—a group of platelets, an eosinophil leucocyte, two polymorphs and two lymphocytes.

Colour correction : the chromatin is somewhat too red and the pigment too bright a yellow ; the mottled blue-grey background of the thick film is not well shown.

PLATE X.
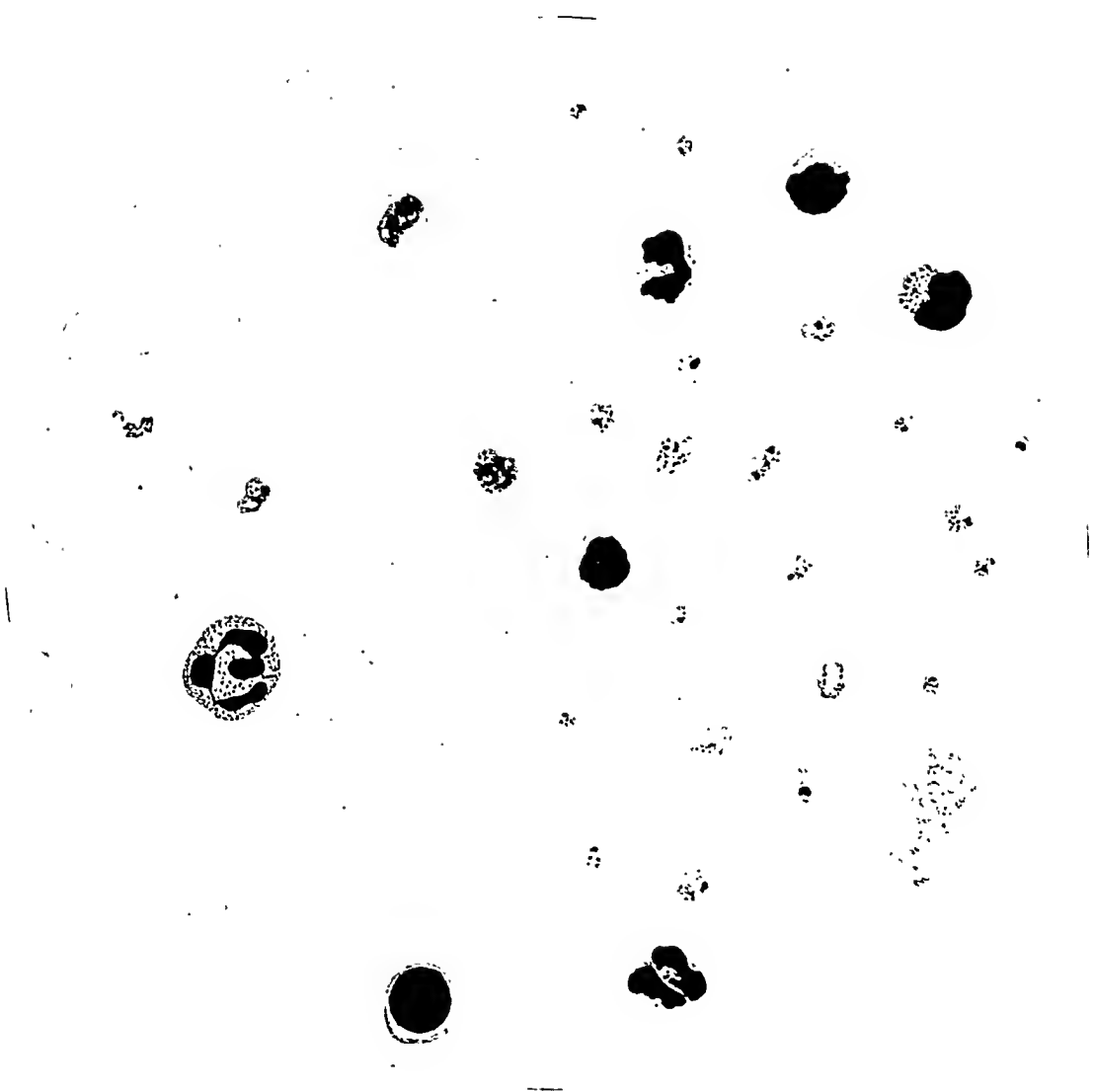
P. malariae IN GIEMSA-STAINED THIN AND THICK BLOOD FILMS.

DESCRIPTION OF PLATE XI.

P. malariae IN GIEMSA-STAINED THICK AND THIN BLOOD FILMS.

FIG. 1.—Young trophozoite—" bird's-eye form "—in thin blood film.

FIG. 2.—Corresponding forms in thick blood film. The host cells have disappeared. The ring formation is usually lost. Note the " comma " like appearance and the large size of the chromatin dot relative to the cytoplasm. The chromatin and cytoplasm are separate and distinct. These forms are not common as the duration of this " comma " stage is not more than a few hours ; they are often associated with mature schizonts. Note that at this stage, and in fact at all stages of growth, the parasites tend to appear smaller in thick films than in fixed thin films.

The photograph also shows two schizonts.

FIG. 3.—Older trophozoite in thin blood film.

FIG. 4.—Corresponding forms in thick film. The chromatin is often obscured by a covering of cytoplasm ; the parasite has become compact and rounded. Pigment appears at this stage. " Rounding off " and pigment formation thus occur very early in development.

The photograph also shows a lymphocyte.

FIG. 5.—Advanced trophozoite—" band form "—in thin blood film at a stage of growth where its future development, whether to a schizont or a gametocyte, is indeterminate. FIG. 6.—Corresponding forms in thick blood film. The " band " appearance is lost. The parasites are rounded and solid looking. The chromatin is often obscured by the cytoplasm. Pigment is now profuse, scattered as granules throughout the cytoplasm, but cannot be satisfactorily demonstrated in a photograph.

The photograph also shows one polymorph.

FIG. 7.—Advanced trophozoites in thin blood film.

FIG. 8.—Corresponding forms in thick blood film. The parasites still tend to retain their compact appearance, and are still ovoid or rounded, but in several there is a suggestion of incipient schizogony. Schizogony is however often delayed until the parasites are approaching full size and at the stage shown it may be difficult to distinguish trophozoites from young gametocytes. Note the apparent smaller size in the thick film.

The photograph also shows one monocyte.
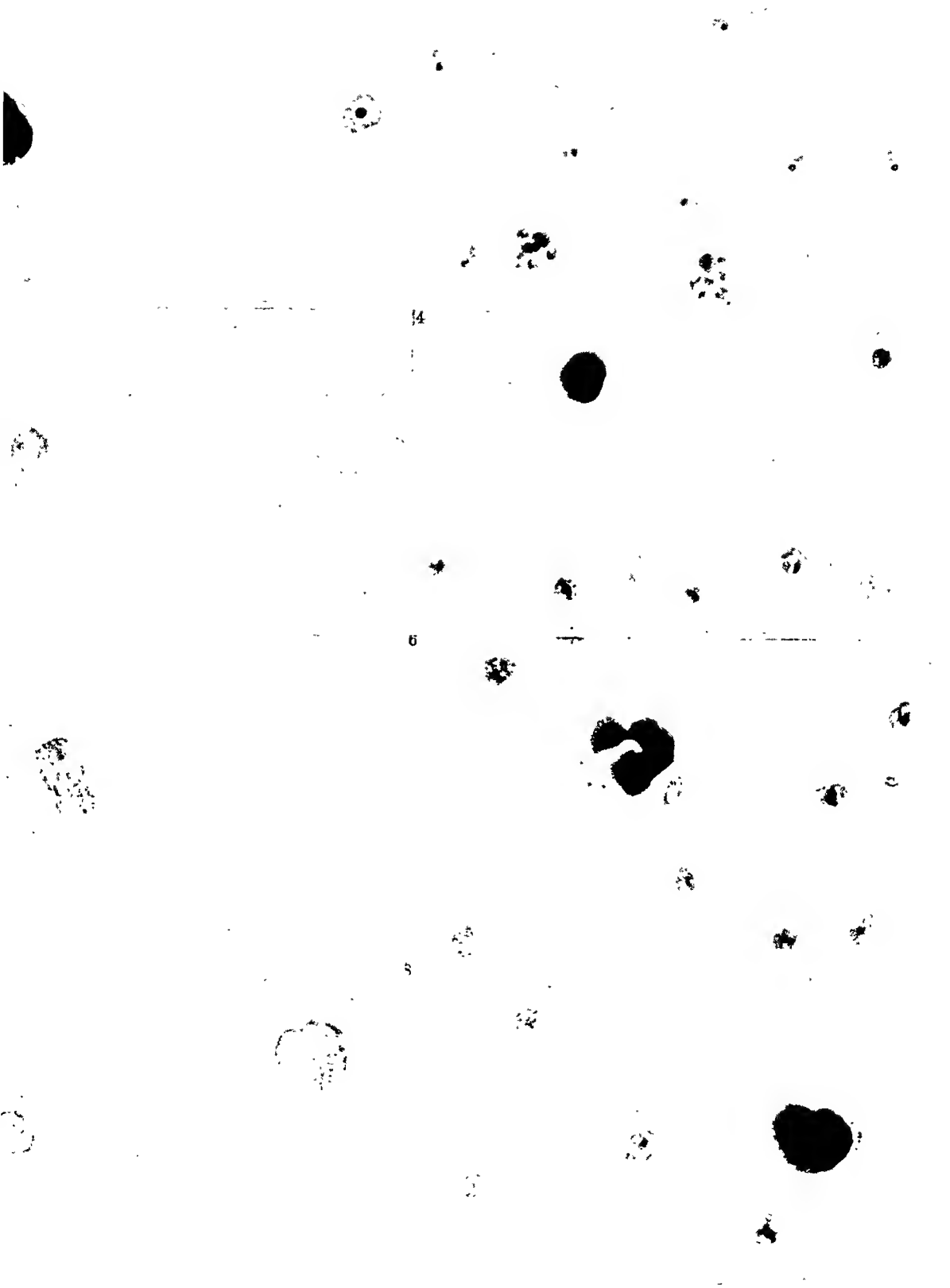
PLATE XI.

CROGRAPHS OF *P. malariae* IN GIEMSA-STAINED THIN AND THICK BLOOD FILMS ($\times$1600 approx.)

Description of Plate XII.

P. malariae in giemsa-stained thick and thin blood films.

Fig. 9.—Early schizont in thin film.

Fig. 10.—Early schizonts in thick film. Chromatin division is now clearly evident. The photograph also shows one eosinophil and one lymphocyte.

Fig. 11.—Advanced schizont in thin blood film.

Fig. 12.—Advanced and mature schizonts in thick blood film. The number of merozoites is characteristically eight, each with an oval intensely staining nucleus. Sometimes the the merozoites show a pale and delicate covering of cyptoplasm but often, as in this figure, the cytoplasm has disappeared during staining and the merozoites appear as unclothed ovoids of chromatin. The pigment is usually concentrated to a single collection but is not so solid and homogeneous as in *P. falciparum* : sometimes it is scattered among the merozoites.

Fig. 13.—Mature schizont in thin blood film.

Fig. 14.—Advanced and mature schizonts in thick blood film. Many of the merozoites still retain a delicate covering of cytoplasm.

The photograph also shows one lymphocyte.

Fig. 15.—Female gametocyte in thin blood film.

Fig. 16.—Gametocyte, probably female, in thick blood film. Gametocytes are easily recognized as such when associated only with schizonts as in the case from which this film was taken, but when found with trophozoites at the stage of development shown in Figs. 6 and 8, identification is difficult.

A mature schizont with a characteristic distribution of pigment is also present.

12

14

16

TRYPANOCIDAL ACTIVITY AND ARSENIC CONTENT OF HUMAN BLOOD AFTER ADMINISTRATION OF TRYPARSAMIDE.

BY

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This paper describes the trypanocidal activity produced in human plasma by the intravenous injection of tryparsamide. By correlating the trypanocidal titre observed with the minimum trypanocidal concentration of a standard trivalent arsenical compound, it is possible to estimate approximately how much of the pentavalent has been reduced into the active trivalent form. It was desired to compare the activity thus produced by tryparsamide in the blood with that which had previously been measured in the cerebrospinal fluid (HAWKING, HENNELLY and QUASTEL, 1937 ; HAWKING, 1940).

Technique.

The experiments were made at Gadau in Northern Nigeria, and the subjects were volunteers from the African laboratory staff. For psychological reasons it was preferable to weigh the subjects *after* the administration of the compound, rather than *before*. The methods used were similar to those employed by MURGATROYD, RUSSELL and YORKE (1934) for similar experiments in rabbits, and by HAWKING, HENNELLY and QUASTEL (1937) for the cerebrospinal fluid.

Tryparsamide was injected intravenously and after suitable intervals, blood was withdrawn for examination and immediately centrifuged. Clotting was prevented by the addition of heparin to a concentration of 1 : 10,000 ; and heparin was also present in the nutrient medium used for dilution. Previous experiments had shown that even a concentration of this particular sample of heparin 1 : 2,500 had practically no effect upon trypanosomes within 24 hours at 37° C. The trypanocidal power of the plasma *in vitro* at 37° C. was determined by the technique described by YORKE, ADAMS and MURGATROYD (1930) ; dilutions were made with a mixture of equal parts of deactivated sheep serum and Locke-glucose solution (nutrient medium), the sera for all experiments being obtained from the same sheep. By setting up a parallel series of tubes containing known concentrations of a standard arsenical compound, as explained in a previous paper (HAWKING, HENNELLY and QUASTEL, 1937) the trypanocidal activity observed was translated into corresponding concentrations of trivalent arsenic. The standard compound used for this purpose was the same as that on previous occasions, *viz.*, $H_2N.CO.CH_2.NH.C_6H_4.As(S.CH_2COONa)_2$—here referred to as reduced tryparsamide thioglycollate. In three of the experiments, the minimum concentration of this compound required to kill trypanosomes of the strain in question within 24 hours at 37° C. was consistently 0·06 γ per ml., *i.e.*, As. 0·01 γ per ml. In one experiment (No. 3), the series of tubes containing the standard compound was unsatisfactory, and in this case the reading is based on that from the other experiments.

Since human plasma is normally trypanocidal for most species of trypanosomes, quite apart from the administration of any drug, it was necessary to use a strain of *T. gambiense*, which is serum-resistant, as a test-object for these experiments ; and the finding and handling of a suitable strain proved the chief difficulty encountered in this work. A strain preserved in the laboratory at Gadau, which produced heavy infections in mice, proved to be highly resistant to arsenicals but sensitive to human serum. Finally, use was made of a strain (Kamianga) from the Belgian Congo which had kindly been provided by Dr. L. VAN HOOF. This was completely serum-resistant and fairly sensitive to arsenicals ; two infected guineapigs treated with tryparsamide in doses of 20 and 70 mg. per kg. respectively became negative for 8 to 11 days (Dr. L. VAN HOOF). Unfortunately its virulence for laboratory animals was low ; mice and rats were not susceptible, many of the subinoculated guineapigs failed to become infected, and it was only at infrequent and irregular intervals that an animal could be obtained, in which trypanosomes were sufficiently numerous to permit an experiment *in vitro* to be made. Owing to the outbreak of war, the time available for this investigation was limited, and consequently it was not possible to repeat and extend these experiments to the extent that would otherwise have been desirable. In all experiments, a sample of plasma from each subject, withdrawn before the administration of tryparsamide, was set up as a control ; in no case was trypanocidal activity of such control plasma upon this strain observed.

Samples of the plasma were sent to Great Britain by air-mail, so that their total arsenic content could be determined chemically through the kind assistance of Mr. S. Dixon, F.I.C., Public Analyst to Cardiff; owing to the same reason —war—it was impossible to do this in every case, as had been originally planned.

Results and Discussion.

The results obtained are shown in the Table, from which it is seen that there is a considerable variation between one individual and another. This was only to be expected. The average of these figures is shown in the graph which also gives the average concentration of active arsenic observed in the cerebro-spinal fluid of persons with approximately normal cerebral membranes, observed
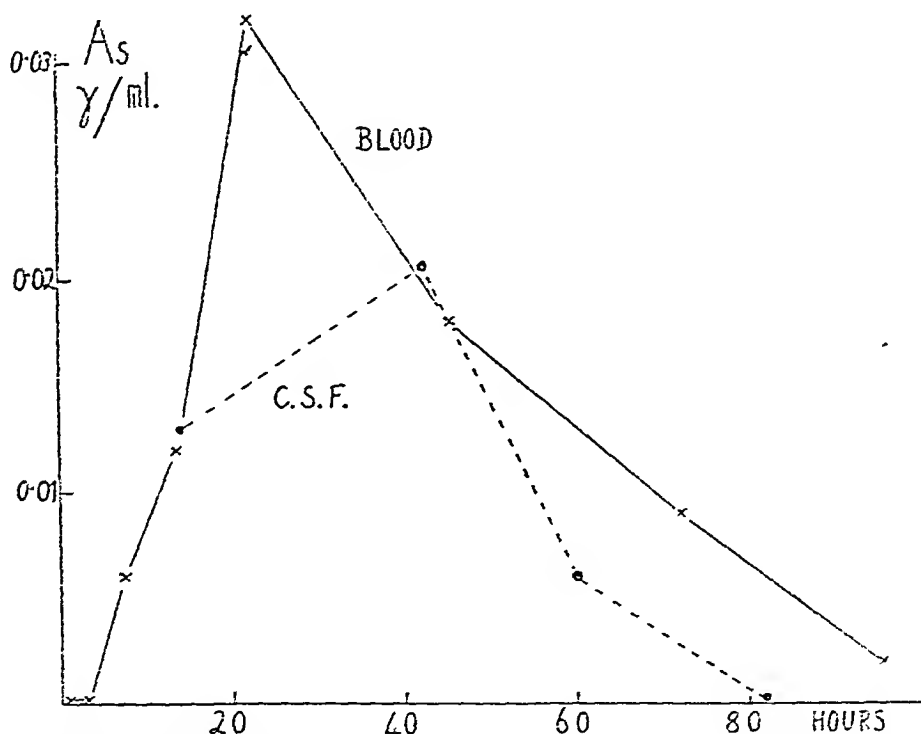
GRAPH.—Showing the trypanocidal activity (expressed as equivalent concentrations of active trivalent arsenic) in the plasma and cerebrospinal fluid after the injection of tryparsamide.

in Great Britain (HAWKING, HENNELLY and QUASTEL, 1937). The curve for trypanocidal activity in the blood is higher than that which occurs in the cerebrospinal fluid; but allowing for the large variation between one individual and another, and for the low degree of accuracy attainable in the measurement of such small concentrations, it is doubtful how far the difference between them is really significant. In each case, activity rises to a maximum from 24 to 48 hours after injection and becomes inappreciable after 96 hours.

TABLE.

SHOWING THE TRYPANOCIDAL ACTIVITY AND ARSENIC CONTENT OF THE PLASMA OF
PERSONS TREATED WITH TRYPARSAMIDE.

Interval. Hours.	Dose. Grammes per 70 kg.	Activity of plasma.		Total arsenic content of plasma. As. γ per ml.	Subject.	No. of experiment.
		Minimal trypanocidal concentration within 24 hours.	Corresponding concentration of reduced tryparsamide. As. γ per ml.			
1	2·6	0	<0·005		A	1
,,	3·0	0	<0·005		B	1
3	2·6	0	<0·005		A	1
,,	3·0	0	<0·005		B	1
7·5	2·6	0	<0·005		A	1
,,	3·0	0	<0·005		B	1
,,	2·5	1 : 1*	0·01		C	2
,,	2·4	1 : 1*	0·01		D	2
13·5	2·3	1 : 1—2	0·015		E	2
,,	3·2	1 : 1	0·01		F	2
21	2·5	1 : 1—2	0·015		C	2
,.	2·4	1 : 2	0·02		D	2
24	3·0	1 : 4*	0·04	0·07	G	3
,,	2·6	1 : 2—4*	0·025	0·07	H	3
,,	2·8	1 : 2*	0·02		I	3
,,	3·0	1 : 2	0·02		J	3
,,	3·5	1 : 4—8*	0·05		K	3
,,	3·0	1 : 8*	0·07		F	3
45	3·0	1 : 2	0·02	0 ?	G	3
,,	2·6	1 : 2—4	0·025	0 ?	H	3
,,	2·8	1 : 1	0·01		I	3
,,	3·0	1 : 1—2	0·015		J	3
,,	3·5	1 : 2	0·02		K	3
,,	3·0	1 : 2	0·02		F	3
72	2·5	? slight action*	? 0·005	0·07	H	4
,,	3·1	1 : 1*	0·01	<0·07	K	4
,,	2·5	1 : 1—2*	0·015		L	4
96	2·5	0	<0·005	0 ?	H	4
,,	3·1	0	<0·005	<0·07	K	4
,,	2·5	? slight action	? 0·005		L	4

* Plasma stored in ice-chest 24 hours.

These results, obtained with human blood, are similar to those recorded by MURGATROYD, RUSSELL and YORKE (1937) for similar experiments in rabbits. In one experiment the rabbit received tryparsamide in a dose of 0·5 gramme per kg. intravenously. The trypanosomes used in their work came from the Liverpool strain of *T. rhodesiense* which is more sensitive to arsenicals than the present strain of *T. gambiense* (minimum trypanocidal concentration of reduced tryparsamide thioglycollate for the Liverpool strain being usually about 0·01 γ per ml., *i.e.*, As. 0·002 γ per ml.). The trypanocidal activity observed rose to a maximum at 6 hours (effective concentration of plasma being 1 : 16, equivalent to As. 0·03 γ per ml.) and then declined gradually (effective concentration of plasma at 24 hours being 1 : 4, equivalent to As. 0·006 γ per ml.), disappearing by the fourth day. In rabbits the trypanocidal activity is more transient than in man.

The chemical estimations of the arsenic content of the plasma were done on samples of only 3 to 4 grammes of material, and the accuracy for these small quantities is therefore low. Nevertheless they indicate that the total amount of the compound which stays in the blood after 24 hours is much lower than might have been expected ; in fact, in these particular persons, the blood concentration was lower than that found in the cerebrospinal fluid and recorded in the previous papers. The amount of arsenic which remains in the blood after the injection of tryparsamide appears to have attracted little attention hitherto, and a partial search of the literature revealed no records concerning it.

In one experiment, preliminary investigations were made by the same method after the injection of neoarsphenamine. One patient received 0·66 gramme per 70 kg. intravenously ; plasma removed one hour later (stored overnight in the ice-chest) had a minimum trypanocidal concentration of 1 : 1,000 equivalent to about 10 γ per ml. of trivalent arsenic reckoned as reduced tryparsamide thioglycollate, or as neoarsphenamine ; plasma removed after 24 hours (fresh) had a minimum trypanocidal concentration of 1 : 32 to 64 equivalent to about 0·6 γ As. per ml. A second patient received 0·58 gramme per 70 kg. ; plasma removed after $1\frac{1}{4}$ hours had a minimum trypanocidal activity of 1 : 500 to 1,000, equivalent to about 7 γ As. per ml. These figures are in agreement with the curves obtained by MURGATROYD, RUSSELL and YORKE (1934) for the trypanocidal activity of rabbit's blood after injection of neoarsphenamine. Further work on neoarsephenamine was prevented by the war.

There is a contrast between the trypanocidal activity produced in the blood and cerebrospinal fluid by neoarsphenamine and that produced by tryparsamide. In the blood, neoarsphenamine causes a high level of activity immediately after injection which then gradually diminishes, being still appreciable for about 10 days (in rabbits, MURGATROYD, RUSSELL and YORKE) ; tryparsamide causes an activity which is absent immediately after injection, rises in about 24 hours to a maximum, which is low compared ·with that for neoarsphenamine, and then dies away within four days. In the cerebrospinal fluid, neoarsphenamine

produces little or no activity (HAWKING, HENNELLY and QUASTEL, 1937); tryparsamide however produces an activity equal to that in the blood, as is shown by the graph on page 307. Apparently the unsatisfactory results observed when neoarsphenamine was used in the treatment of human trypanosomiasis were due to its failure to penetrate into the nervous and (probably) other tissues.

SUMMARY.

1. Persons were given an intravenous injection of tryparsamide and measurements were then made of the power of the plasma to kill trypanosomes of a serum-resistant strain of *T. gambiense*.

2. Immediately after the injection, trypanocidal activity was absent ; it rose to a maximum at 24 hours, at which time the minimum trypanocidal concentration (24 hours' exposure *in vitro* at 37° C.) varied between 1 : 2 and 1 : 8, corresponding to about 0·03 γ per ml. of trivalent arsenic. The activity then gradually diminished and 4 days after the injection it was inappreciable.

3. Judging by a limited number of chemical estimations of the total arsenic content, tryparsamide disappears from the blood very rapidly after injection ; in two persons the arsenic content of the plasma, 24 hours after injection, was only 0·07 γ As. per ml.

4. The trypanocidal activity produced by tryparsamide in the blood was somewhat greater than that previously observed to occur in the cerebrospinal fluid, but its time-relations were approximately similar.

5. Preliminary experiments indicated that neoarsphenamine produces a much greater activity in the blood than tryparsamide does, the minimum trypanocidal concentration of the plasma one hour after injection being about 1 : 1,000 (corresponding to 7 to 10 γ per ml. of trivalent arsenic) ; after 24 hours this activity diminishes, so that it corresponds to 0·6 γ As. per ml. These results confirm those obtained by MURGATROYD, RUSSELL and YORKE, working with rabbits.

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A MALARIA SURVEY ON THE CHINA-BURMA HIGHWAY

BY

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INTRODUCTION.

The China-Burma Highway has featured very prominently recently in the sphere of Far Eastern politics and the road has a great future for commercial transportation when the present needs for a munitions supply route cease.

Little information is as yet on record as to the history of the construction of the road or the pioneer medical work which has been accomplished in order to render the notorious malarial districts traversed safer for the transport workers. There is no question but that this new line of communication between Burma and China has already served a vital need during China's war of resistance. and its usefulness is bound to grow as time goes on.

The excellent facilities of Rangoon as a deep-water port, the network of fine motor roads and railways in Burma and the extensive navigation system of the Irrawaddy River are now all within reach of China's richest provinces. It is obvious that once a trade route has been established it will not be permanently closed by present military or political exigencies.

The Construction of the Road.

The proposal to connect Burma and China was put forward as early as 1860; attempts to explore the possibilities of improving the old caravan routes between Bhamo in upper Burma and western Yunnan were made by Col. E. B. SLADEN in 1868, Augustus R. MARGARY in 1874 and Major DAVIS in 1895. Before Burma had her present railways and roads, the country was well opened up as far as Bhamo through inland navigation by the famous Irrawaddy Flotilla Company.

MAP A.
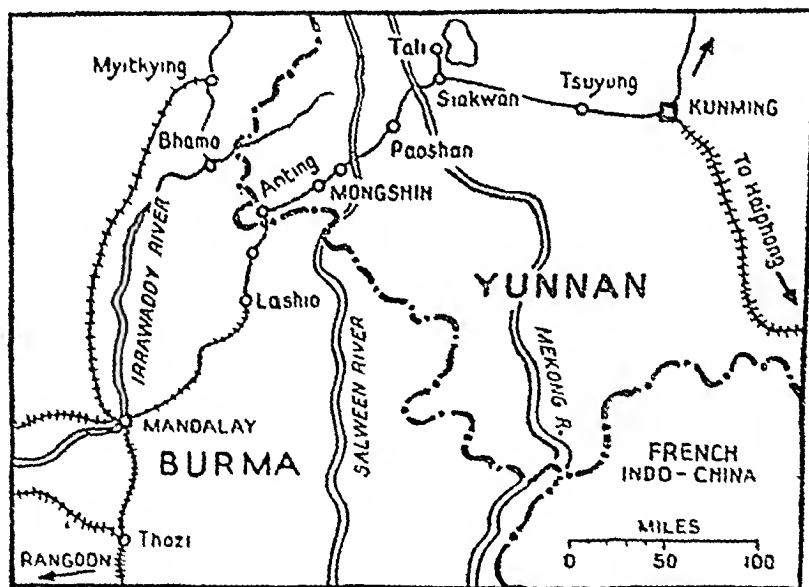
Map of Yunnan Province showing Burma Road and Yunnan Railway,
also Positions of the great Rivers.

It was not, however, till the outbreak of the Sino-Japanese war in 1937 that the Chinese Government became alive to the necessity of constructing a modern motor road from Kunming to the Burma border. The closure of the ports on the Pacific coast by the Japanese invasion accentuated the need for this artery of communication. It is true to say that 40 per cent. of the Yunnan-

Burma Road had been built before the Lukouchiao incident; and by July, 1938, the eastern section of the older Highway between Kunming and Hsiakwan had been widened and surfaced for motor traffic. The western section from Hsiakwan to the border was opened for light traffic in the beginning of 1939. This new construction was carried out with phenomenal rapidity though the difficulties which had to be surmounted were enormous. The road has to cross two great rivers which cut deep canyons into the high Yunnan Plateau. In each case a descent had to be undertaken, with the use of elaborate hairpin bends, from an altitude of between 9,000 and 10,000 feet above sea level to about 3,000 feet, where one crosses the Mekong and Salween by suspension bridges. Then the road beyond the valley of the Salween descends by more gradual stages to the notorious swampy valleys in the Chinese Shan State. These valleys have been known for centuries to be death traps owing to malaria (chang-ch'i). From time immemorial, it has been the custom of the local Chinese inhabitants in the most malarious districts on the plain to migrate towards the mountains at the onset of the rainy season in June and return again to the valleys at the end of October leaving only a scattered farming population to deal with the cultivation of the rice paddies. The construction of the new road has changed many of these established customs. Large numbers of Chinese transport workers have come into the region. Depots for motor trucks, fuel supplies, repair shops and ammunition dumps have been constructed. Labour has been mobilised to deal with the repair and upkeep of the road. The local Shan people have been attracted to stay on in their villages on the plain to supply the market needs of this new influx of population during the rainy season. In addition to these factors, an army of motor drivers is constantly passing to and fro with the hundreds of lorries used for transport.

Owing to the rapidity with which the road bed was constructed, particularly in the western section, insufficient attention was paid to road drainage, and often in the wet season torrents come down the mountain side and wash away the road. Landslides are of frequent occurrence and the road may be blocked for days. Accidents are very numerous owing to the soft muddy nature of the road and the precipitous descents. At places the road is literally a soft crumbling ledge cut out of the face of a cliff and many trucks have been hurled to the ravines below with certain death to the drivers and passengers. Blockages on these difficult parts are very frequent. The result of this is that in the summer of 1939 convoys of transport were held up for days on end, sometimes in very remote places. The drivers had to sleep in the wagons with no protection against mosquitoes and often with no food available for several days. It is therefore readily understood that malaria is rife among these transport workers. Following the lessons learnt in the 1939 rainy season some improvement has takn place and it was hoped that in the summer of 1940 the road would be kept open throughout the monsoon season.

Unfortunately the various depôts on the road have been chosen for geographical and other reasons not connected with epidemiology: this is a great mistake—out of all proportion to any advantages—and one that is being, and will have to be, paid for in ill-health of the community due to malaria.

Physical Feature of the Route.

A journey over the Yunnan-Burma Highway is a fascinating experience. The countries traversed present a great variety of scenery and a wonderful display of colour, particularly Upper Burma with its magnificent forests, flowers and wild animals. The same is true of the Shan states with an interesting people, a fine race embodying many of the best qualities of the Chinese with the genial nature of the Burmese. Then one crosses two mighty rivers along the road—the 800 mile long Salween that flows into the Gulf of Martaban near Moulmein, and the 2,800 mile long Mekong which has its source in the Tibetan Highlands and flows through Yunnan. Burma, Siam, Cambodia and Cochin-China before it empties into the South China Sea.

The valleys of these great rivers are really deep gorges or canyons cut through the Yunnan plateau. The New Road descends into these canyons by terrific hairpin bends and one climbs the other side by a similar road skirting the awe-inspiring precipices. The engineering difficulties which have been overcome are stupendous; and one rightly considers that in pushing through this Highway the Chinese have accomplished a feat which compares in importance with the construction of the Great Wall of China over two thousand years ago. The Great Wall was erected to ensure China's isolation, the Burma Road to give an outlet to the west. Only Chinese pertinacity and perseverance, with a complete disregard for the countless human lives sacrificed could accomplish this feat. Modern rock-cutting machinery and other mechanical aids to road construction were not available.

Along the Yunnan-Burma Highway, particularly between Lashio and Mangshih there are as many as sixty different aboriginal tribes. Some look very much like Chinese, notably the Kachins, the Shans and the Payis; but others are more strange looking, especially the primitive mountaineers. One finds gangs of these people at work on the Highway carrying medieval weapons and wearing picturesque clothes.

Yunnan Province, which is entered at Lungling, is one of the most interesting provinces of China. "Yunnan" signifies "South of the Clouds" and one has to climb 9,000 feet about sea level to reach the high table-land. The scenery is very mountainous and the climate on the whole healthy. The soil in the small valleys between the mountain ranges is fertile and the crops are usually good. Between Tali and Tsouhsiung, for example, one can get oranges as big and delicious as the famous Californian "Sunkist" variety. Tea crops are also good in the Shunning district. Yunnan is famous for its mineral

1.—The China-Burma Road passes over high mountain ranges.
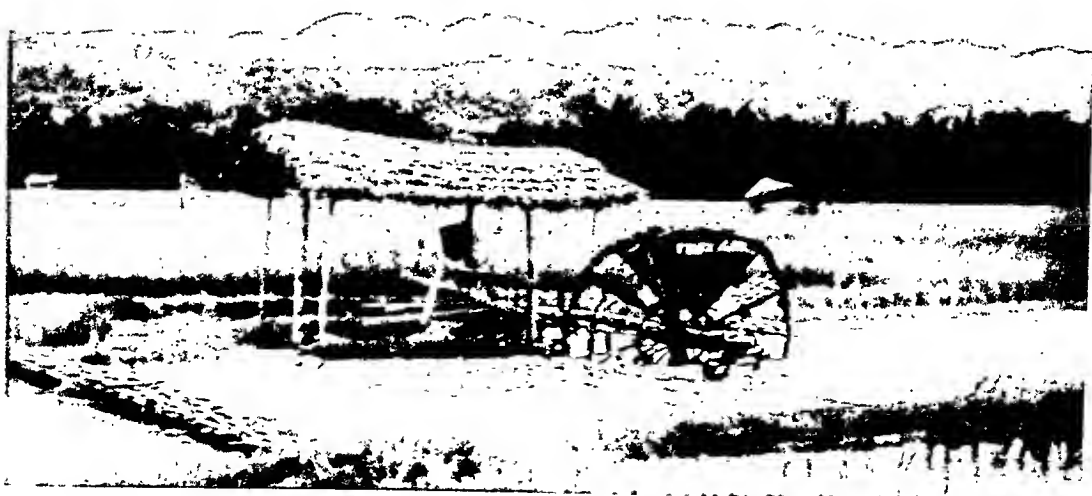
2.—The low lying plains are swampy and malarious. Extensive rice cultivation
is carried out.
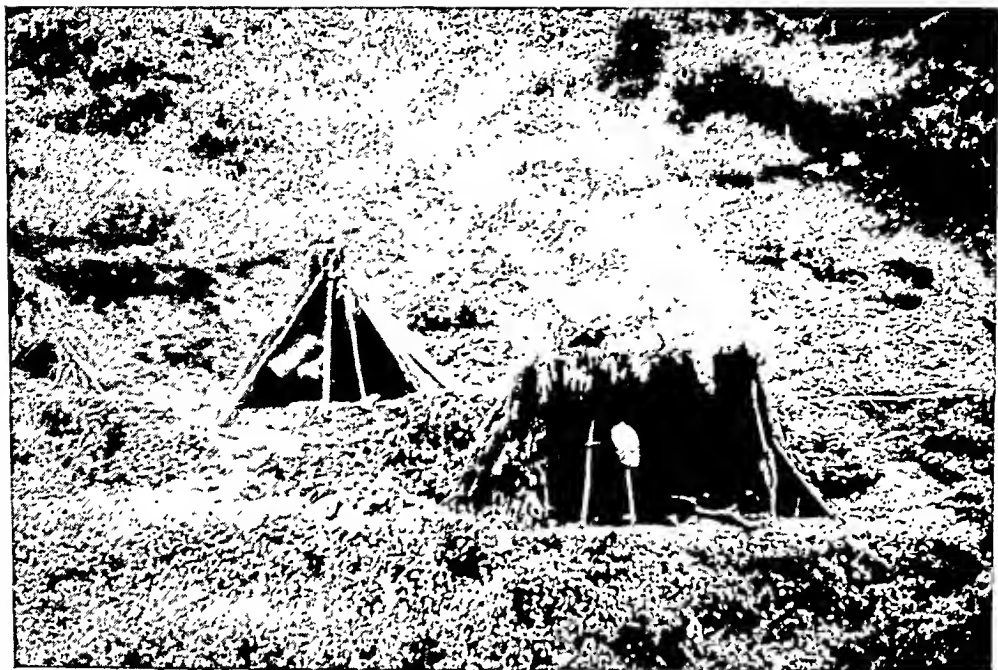
3.—Primitive shelters made of bamboo and branches of trees are used by the road workers.

4.—Clearing away a landslide on the Burma Road.

5.—The villages are unsuitable for the new transport depots.

6.—The Shan villagers hold open air markets. The women wear brimless top hats.

7.—Rock salt carriers. Goitre and cretinism are very evident on the Burma road.
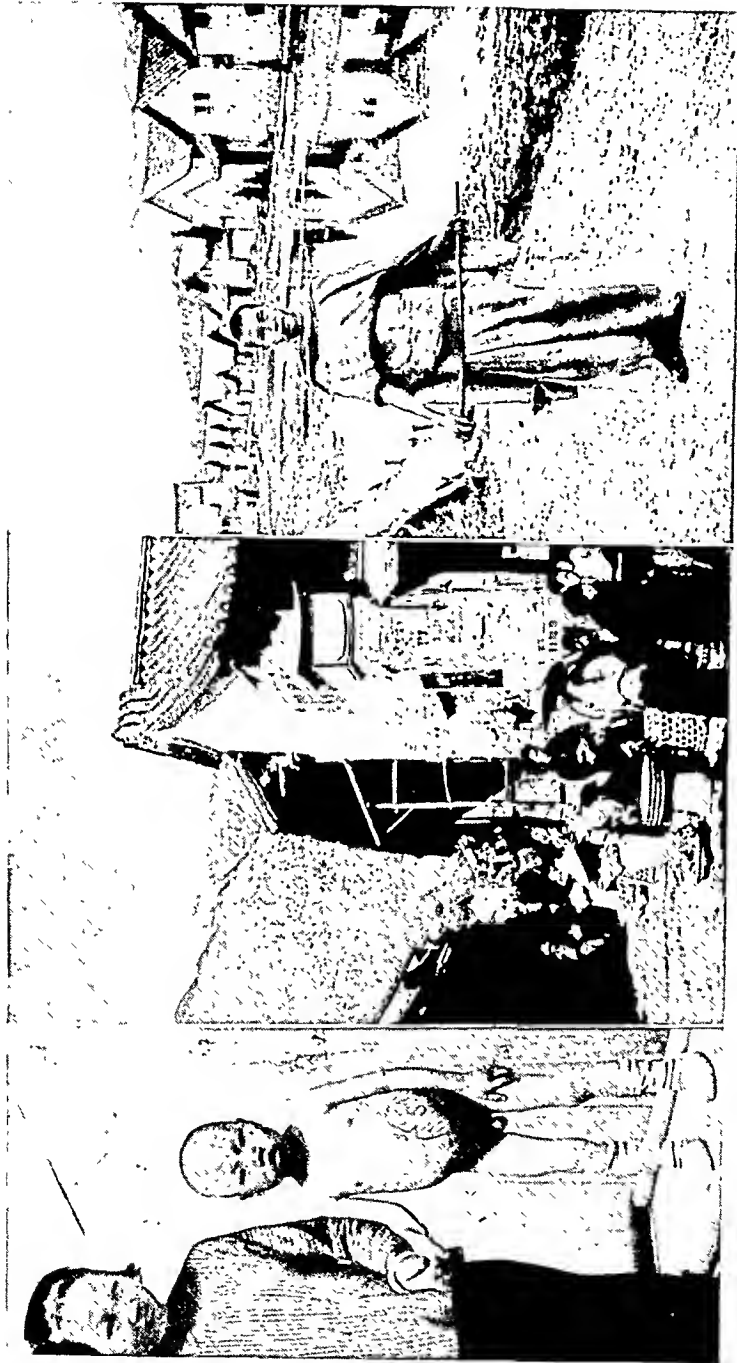
8.—Chinese child with splenic enlargement due to malaria.

9.—Hsiakwan near Talifu. The scenery resembles Switzerland.

10.—A Shan village in the endemic malaria region at Mangshih.

wealth: tin, antimony, coal, copper, gold, iron, mercury, silver and tungsten. Many of these natural resources remain undeveloped. Even the most casual visitor to Yunnan Province cannot but be impressed with the extremely high incidence of thyroid enlargement. In many cases it is very evident that along with the thyroid enlargement, which is endemic goitre, there are obvious symptoms of cretinism.

The adult population, both men and women, working on the road show in some gangs an incidence of goitre as high as 80 per cent. The average among the working gangs is well over 50 per cent. This goitre problem was one of the chief medical features, second only in importance to malaria, which occupied my attention during the preliminary surveys along the new Highway. The endemic goitre of Yunnan is due to the lack of iodine in the soil, water and vegetation; and a scheme for amelioration of the condition has been put in hand by artificially iodising Yunnan domestic salt at the local mines. In this province "iodine days" have also been instituted for the population on quite an extensive scale: this consists of giving at suitable intervals the minute amount of potassium iodide required to maintain thyroid equilibrium.

The south-western part of Yunnan is the most diverse in China in the constituents of the population. About half are true Chinese and the remainder are made up of many varied tribes of other races, chiefly Miao, Lolo with Tibetan and Burmese stocks in the extreme west. The Shan (that is "Highland") people are simple and ignorant but strong and energetic. The territory occupied by these races in the west has a semi-independent political status. The authority of the Chinese Government being effective only as far as military control exists and chiefly on the fringes of the new Highway. The Shans are governed by hereditary Chieftains for all domestic concerns. Much the same type of government is to be found in the neighbouring British Federated Shan State.

As a result of this condition of affairs the boundary between China and Burma is merely a political one: there is really no social frontier, as the people of the Shan States go to and fro without restriction: they are of the same race and customs. The monetary system is based on the Indian silver rupee and subsidiary coinage. The old Yunnan silver dollar is also used along with provincial copper coins. In China proper the silver national dollar has disappeared from circulation.

MALARIA SURVEYS

It is most convenient to consider the China-Burma Highway from the standpoint of the malariologist as being divided into three parts (1) a region of slight malaria prevalence from Kunming to the canyon of the Mekong, (2) a region of patchy malaria prevalence, rather intense in certain remote valleys

and absent in the mountain ranges till Lungling on the border of the Shan State is reached, (3) a region of severe endemicity stretching from Lungling through Mangshih to the frontier. The part of the road from the frontier post at Wanting through the British Shan State to Lashio, the railhead, is again patchy. Lashio itself is fairly healthy and a certain amount of control work has been carried out there by the Burmese Health Services during past years. The great influx of a new Chinese population since the opening up of the road is liable to alter conditions. The new Chinese transport depôt at Lashio has numerous cases of malaria, but it is at the moment difficult to obtain facts as to the amount of original infection in Lashio itself.

The survey has had to take into account the different population groups concerned and as there is a large transitory population on the Highway certain extremely important qualifications are necessary before a just estimate can be made of the exact prevalence of malaria in any one district. Attention is chiefly directed to the worst malarious districts in the Chinese Shan State.

The population groups studied are as follows:—

(1) The local Chinese inhabitants who are chiefly to be found in Lungling and Monkar districts.

(2) The local Shan inhabitants.

(3) Tribesmen, chiefly Bonlung, Loosu, Miao, who are in Chafeng district and come down to the markets on the Burma Highway from the hills.

(4) Chinese population of the "colonial" type—officials and their staff belonging to the various governmental organisations.

(5) Chinese transient population; transport workers, drivers, road repair gangs.

(6) Burmese transient population: transport workers, merchants, travellers.

The anti-malaria work which was undertaken during 1939 centred around a number of medical clinics established by the South-Western Transport Bureau, the Yunnan Highway Bureau and two mobile units of the National Health Service called anti-epidemic teams. These last were supported by the League of Nations Epidemic Commission. For survey purposes I established a mobile laboratory in the first instance at Mangshih. This laboratory was principally concerned in making blood-film examinations and in collecting entomological data.

In a paper published in the *Chinese Medical Journal* (ROBERTSON, 1940) I have described some of the general features of the malaria situation along the Highway. In the present communication I propose to deal in more detail with the most severely affected region which stretches from Lungling through Mangshih to the Burma frontier.

Region of Severe Malarial Endemicity

Topography.

The town of Lungling and the County of Lushih lie on the western portion of the new Highway. The canyon of the Salween River must be crossed before entering this region from the eastern sector. The Shan State under Chinese administration is entered west of Lungling, where there is a customs station under the Chinese National Government Customs Service.

The district lies between latitude 24°-25° N. and is bounded by longitude 98°-99° E. The town of Lungling lies on the slopes of the Kao-li-kung mountains. From this point the road runs south-west to the border, descending abruptly 1,000 feet to one of the first swampy plains. It may be said that this swampy plain is divided longitudinally by a mountain range called Santai Shan. The eastern portion is the Mangshih area and the western Chefang. There is a higher plain to the south of Lushih County in which the town of Monkar is situated. This forms a special district.

Population.

Although Lungling and the villages or townships of Lushih County are in fairly close proximity, the races inhabiting the districts are very diverse.

In Lungling we have a typically Chinese population predominating, with a scanty admixture of tribesmen, whereas in Lushih County there is a very mixed population with all grades of civilization. Less than one-third of the inhabitants are Chinese, half are Shan people and the remainder primitive tribesmen.

The Chinese are essentially immigrants from other provinces and have migrated to what have been considered through the years to be the less malarious districts. These are the upper plains of the table-land and the slopes of the high mountains. The fear of "*chang-ch'i*" (malaria) has played a great part in the distribution of the population within historical times. We find that Lungling and Monkar are chiefly Chinese towns.

As there is very limited agricultural land on the mountain slopes, the Chinese population have had to depend on rice and other foods imported from the neighbouring towns. The plains are very fertile and suitable for agriculture but they have been avoided for settlement by the Chinese on account of their evil reputation for malaria.

The Shan people occupy the fertile plains of Mangshih and Chefang in Lushih County. They have their own language and customs which are entirely different from Chinese. They lead a semi-independent political existence. In character, they are simple and ignorant, having until recently been cut off from the rest of the world by difficult lines of communication. They are, however, cheerful and kindly in disposition, although very conservative as regards their own native customs.

The primitive tribes live in the more remote districts in the mountains and eke out a simple living as herdsmen, hunters and small farmers. They are generally known as Bonlung and Loosu, and resemble the Miao tribes of other parts of China. These tribesmen avoid contact with the Chinese as much as possible and are chiefly seen on market days in small bands when they come down to sell their products or exchange firewood for foodstuffs and alcoholic liquor.

The density of the population is not great and one finds considerable tracts of arable land on the plains used only for cattle grazing. Malaria has played a considerable part in keeping down the population of the fertile plains of the Shan states and has kept back Chinese immigration for many generations. The housing conditions are very primitive: most of the dwellings are constructed of bamboo with mud walls, and they are readily entered by mosquitoes through many openings.

The rooms in the houses are used for sleeping as the daily occupations are conducted in outdoor shelters, or in the ancestral room in the better built houses. These bedrooms are invariably small, dark and stuffed with furniture. Mosquito nets are used but are very badly kept and full of holes. Social education in this respect might do a great deal to keep down malaria prevalence. Further, the agricultural habits of the people have influenced the grouping of the settlements and villages. These are clustered around streams and springs as water supply is the main consideration for rice cultivation and its availability in the dry season determines the place where villages have developed.

Climate.

The climate is temperate with no great extremes of heat or cold. In summer the temperature rarely exceeds 85° F. and in winter snow and ice are unknown.

The dry season begins in November and continues till the end of April: during these months there is a great deal of sunshine and the climate is very delightful. In the monsoon season the rains are almost continuous with brief intervals of hot sunshine. At times the rains are torrential and the rivers rapidly rise and flood all the low-lying land in the vicinity. The amount and distribution of the rainfall has a great influence on the various types of breeding places of the local anopheles.

Cultivation in Relation to Mosquito Breeding.

In this particular area the chief breeding places of the anopheline vectors of malaria are (1) bodies of stagnant water, (2) slow or fast moving streams. Both of these are affected in volume and distribution by the summer rains.

Rice is the chief summer crop and the state of the paddy fields has a distinct bearing on malaria prevalence as the season advances. Rice is sown

in seed beds in April, transplanted in May, and ready for harvest in September and October. The fields remain under water for four to five months of the year. Winter crops are not so common. Fields may be found planted with wheat, beans or oil seeds.

The cultivation of opium has long been important in this part of the country but within recent years it is restricted to regions distant from the Highway. Opium smoking is, however, very prevalent and the drug is easily obtainable.

This region is plentifully supplied with domestic animals. Cattle, goats, pigs and ducks are very numerous, while horses, mules and cows are used in transportation. Most natives keep pigs and poultry.

PREVALENCE OF MALARIA.

Lungling and the adjacent County of Lushih have had an evil reputation for *chang-ch'i* (which is really subtertian malaria) for many years. Fear of *chang-ch'i* is deeply rooted in the minds of the people and the notoriety of this district has spread far and wide in China. This fear of malaria is well expressed in common sayings and on the inscriptions on tablets in the temples. One of these is the well known saying: " If any traveller wishes to cross the Salween River, he is well advised to make his last will and testament, leaving permission for his wife to marry again." Undoubtedly the valley of the Salween was regarded as the barrier to a demon-infested district from which travellers in the old days rarely returned alive. Naturally, besides physical difficulties presented by the inaccessible mountain ranges and the malarious swamps, the region was given a bad reputation by Chinese travellers. The construction of the Highway marks a new epoch with the influx of large numbers of transport workers and labour gangs. A new problem has arisen which taxes all available resources in modern sanitation and the science of malariology.

It is necessary to realise that the new immigrants to the district have to live in unsuitable houses; whilst the labour gangs for the maintenance of the road use huts made of bamboo, branches of trees and leaves, and sometimes shelter in primitive caves by the roadside. This presents an increasing difficulty in instituting organised malaria control.

In order to gain an idea of the prevalence of malaria in this area, an inspection of hospital records was made in Lungling and Mangshih. Among 1,392 patients, chiefly local inhabitants and transport workers in the district, who came for attention to the Lungling Health Centre for various conditions during August, September and October, 1939, 546 or 39·2 per cent. were suffering from malaria.

A similar examination was made in Mangshih. There are two clinics, one supported by the South-western Transportation Bureau and the other attached to the China-Burma Highway Bureau. The former chiefly deals with transport

workers, drivers of cars, etc., and the latter looks after the Highway coolies and officials of the Bureau. Of 1,412 patients who came for medical attention to the South-western Transportation Bureau Clinic, 1,012 or 78·0 per cent. were diagnosed as malaria cases from July to September, 1939. Out of 4,203 cases coming to the Highway Bureau Clinic for various conditions during the same period, 1,989 or 47·3 per cent. were suffering from malaria.

There are marked differences in malaria incidence among these hospitals or clinics outside the region we have surveyed. Paoshan, a town on the China-Burma Highway, adjacent to Lungling but separated by deep canyons of the Salween, reports a malaria incidence of only 8·2 per cent. for 3,062 patients who came for attention to Paoshan Health Station from July to October, 1939. Hsiakwan, another town on the mid-way of the China-Burma Highway reports 13·5 per cent. of 1,121 patients as suffering from malaria as seen in the clinic attached to the Highway Bureau during the same period. GEAR (1936) gives an incidence of 2·5 per cent. malaria patients admitted to the C.M.S. Hospital in Kunming.

We think it is very necessary to make it quite clear what population groups we have studied, as statistical figures on malaria are very liable to misinterpretation unless this is definitely stated. It is reasonable to believe that figures gained from the study of permanent residents will differ greatly from those obtained from a new " colonial " population. Then when a region is recently opened up or involved in an influx of new population such as we are dealing with here, any figures are very liable to fluctuation. We considered it best to decide on taking blood smears for statistical purposes from native children under 14 years of age. The Shan people and the native tribesmen are very difficult to approach and they resent anything in the way of systematic medical examination. We also found the language a serious hindrance to the work, which was of a distinctly pioneer character.

At the same time we had no lack of material from Chinese patients to deal with and, of course, the *raison d'être* of our work was malaria control amongst the population using the new Highway for transport, or the staff of road workers and so on.

The statistical information gathered from this transient or " colonial " group presents quite a different picture from that gained from the permanent native population. Table I, page 321 represents the restricted group of native children mentioned above.

Both thick and thin smears were utilised for our blood examinations. Palpation of the spleen was made in the erect position, the clothing being removed from the abdomen.

FEEGRADE (1926) reports that in Lashio he found a parasite index of 16·4, whilst at Bahmo a small series of school children gave 6·7 per cent. of infection. He records spleen rates from 0 to 33 in different areas studied in the British

Federated Shan States. These are the only comparisons we can find in the literature regarding territory adjacent to the region we have surveyed.

There have been very few scientific observations in the past on the malaria of this province. We have to depend on the records of LING, YAO and LIU (1936) for a region in southern Yunnan. From their studies of *chang-ch'i*

TABLE I.

Locality.	Number of Children Examined.	Blood.		Spleen.	
		Number Positive.	Percentage.	Number Palpable.	Percentage.
Lungling	286	46	16·0	17	9·4
Mangshih	163	37	22·0	35	21·4
Chefang...	54	8	14·8	7	13·0
Hwapa ...	105	19	18·1	33	31·4
Namow ...	93	17	18·2	28	30·1
Monkar ...	264	55	20·8	48	18·2
Total ...	965	182	18·9	168	17·4

(malaria) districts we find that the parasite index ranges from 7·66 to 68·17 and the spleen index from 12·05 to 100·00. The average parasite index is given as 27·40 and the average spleen index as 49·75.

TABLE II.

TYPES OF MALARIA PARASITES IN 1057 BLOOD SMEARS.

	Number.	Percentage.
P. falciparum	759	71·8
P. vivax	266	25·2
P. malariae	32	3·0

With regard to the above-mentioned findings as to the relative frequency of the different types of malaria, 1,057 positive blood smears were examined and it is obvious that sub-tertian malaria is by far the commonest infection. The source of this material was our collection of positive blood smears from each village in the survey district. No particular population group was selected, but in order to determine the relative frequency of the different types of malaria in each village studied we selected only films from natives who had not migrated from other places.

It is evident that sub-tertian malaria is prevalent in the low lying plains such as Mangshih, Chefang, Hwapa and Namow. In the upper plains of high altitude such as Lungling and Monkar, we find benign tertian the prevailing infection. Quartan in any case is rare in this region, except in one town, Hwapa, where 31·2 per cent. of the total blood smears examined were found positive for *P. malariae*.

It is very probable that this distribution of the various types of malaria will be subject to change in the near future due to improvement in means of communication between the villages and centres of population. Side-roads are

MAP B.
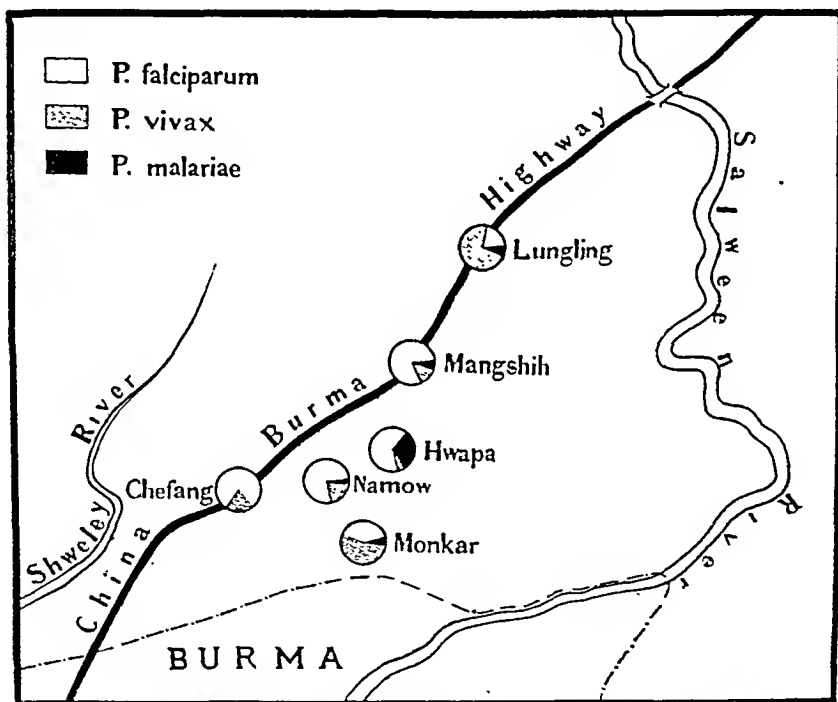
Types of malaria parasites found in blood smears.

being constructed to facilitate access to the new Highway. When large numbers of Highway construction coolies come into the remoter districts they will be followed by transport workers and other members of the "colonial" population group. These will probably bring malaria parasites from the plains and infect the local anopheline vectors. It will, however, be interesting to see what happens. From our data we found that in Lungling the sub-tertian cases had been infected chiefly in the low plain before coming up to Lungling. Again during the lengthy monsoon rains, Monkar was isolated from the Mangshih

plain by floods and we then found the fresh cases of malaria to be benign tertian. It would be interesting to discover if sub-tertian malaria is definitely a disease of lower altitudes than is benign tertian or quartan malaria. We have so far no conclusive facts which would inculpate any particular vector as being specially a carrier of sub-tertian malaria. We know that *Anopheles minimus* and *A. hyrcanus* var. *sinensis* can both act as vectors for any of the types of malaria, though it is certainly the case that where *A. minimus* and other closely allied species are abundant we usually find sub-tertian malaria to be common.

Table III shows the relative infectivity among different races of people in this region, as well as the relative incidence of different types of malaria prevailing among them. There does not appear to be very much difference in the malaria infection rate of Chinese and Shan people. The percentage of infection given for other tribesmen is hardly valuable for comparison as the number examined is too small. It is very difficult to gain access to these tribesmen on account of their uncivilised habits and the language difficulty: of 21 examined, one was positive for *P. vivax*.

TABLE III.

Race.	Number Examined.	Type of Malarial Parasite.						Per cent. of Infection.
		P. vivax.		P. falciparum.		P. malariae.		
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Chinese ...	550	53	52·4	39	38·7	9	8·9	18·3
Shan people	394	9	10·0	71	78·8	10	11·1	20·4

With regard to the difference in the type of malaria prevalent amongst the Chinese and Shan people, the explanation is simple as the greater number of the Chinese live on the upper plains where benign tertian malaria is commoner. When the Chinese live in the sub-tertian districts they are just as liable to this infection as are the Shans. In fact the new Chinese immigrants usually take malaria in a more malignant form.

The Chinese speak of *ya-chang* or dumb *chang-ch'i* which affects newcomers more severely than the Shan people who are partially immune to malaria from repeated attacks. This *ya-chang* is malignant sub-tertian malaria which present symptoms of coma, severe vomiting and sometimes diarrhoea. *Ya-chang* or cerebral malaria is common among the Chinese of the "colonial" type also amongst the chauffeurs and transport workers who have been recruited from abroad: Straits Chinese, Hongkong Chinese and Chinese from the northern provinces. On account of the dread of *ya-chang*

the native Chinese have the custom of migrating to the higher plains or mountains during the rainy season and returning when the rains cease in October.

Towards the end of the monsoon rains we noticed an increased prevalence of these severe malaria cases amongst the Chinese on the Highway. The available hospital beds in the detention hospitals attached to the various clinics were chiefly occupied by such serious cases of malaria. Intravenous quinine therapy had to be largely used in the treatment of these patients: they were quite beyond being benefited by the ordinary oral administration of quinine tablets.

ENTOMOLOGICAL DATA.

Yunnan has hitherto received scant attention as regards its mosquito fauna. This has been chiefly due to the difficulties of travel in the province up to the establishment of the Burma Highway in 1939. Such surveys as have taken place in the past have been chiefly along previously established routes of travel. GASCHEN (1934) conducted an entomological survey along the French railway from Lao-kay to Yunnanfu (Kunming) and found nine species of anopheles in the region he visited. YAO and LING (1937) made a study of the mosquito fauna of South-Western China and gave a list of fifteen species of anopheles for the Province of Yunnan. The regions visited comprised the southern parts of the province and some stations along the railway to Indo-China.

In connection with the survey reported in this communication I was fortunate to have the services of Mr. CHANG TEH-LING, B.S., who, along with a small technical staff, was attached to my mobile survey unit. Mr. CHANG had been for a number of years on my staff in the Entomological Laboratories of the Henry Lester Institute, Shanghai, and was specially seconded by the Institute for my personal technical staff on the League of Nations Anti-Epidemic Commission.

I established the entomological laboratory in Mangshih as a centre with motor-van transport and travelling equipment for extended surveys. We operated on the China-Burma Highway from April to October, 1939 A further survey was made during the monsoon season in the low lying plains in the Mangshih and Chefang areas and for one month on the upper plateau region at Lungling. It may be of interest to mention at this juncture that a small group of malariologists was sent out by the United States Government Health Service on a mission to South-Western China in Nevember, 1939. This group, led by Dr. L. J. WILLIAMS, operated during January, February and March, 1940, in the Chefang region. When the results of their observations are published we shall have a fairly complete record of the distribution of anopheline mosquitoes throughout the year on this part of the China-Burma Highway.

Table IV shows the distribution of different species of *Anopheles* in the towns and districts surveyed. The identification of species was based on

collections of larvae and adults as well as on specimens reared from the larval stages in the laboratory. Brief notes were recorded on the breeding habits as well as on the adult behaviour of most of the species found.

There were altogether seventeen species and varieties of *Anopheles* collected at different places visited in Yunnan. *A. jamesi* and *A. tesselatus* have not previously been reported in this province. *A. splendidus* which was found in Sze-mao by YAO and LING (1937) has not been found in any of the districts visited by us, and thus the total number of species and varieties of *Anopheles* definitely recorded in this province consists now of eighteen.

TABLE IV.

Species \ Locality	Kunming	Ipinglong	Yuenyungching	Chuyung	Hsiakwen	Yungping	Paoshan	Lungling	Mangshih	Chefang	Chachia	Hweitseh	Monkar
A. aitkeni													
A. lindesayi var. (?)													
A. gigas var. baileyi													
A. hyrcanus var. sinensis													
A. hyrcanus var. nigerrimus													
A. barbirostris													
A. kochi													
A. tessellatus													
A. culicifacies													
A. minimus													
A. jeyporiensis var. candidiensis													
A. vagus													
A. maculatus													
A. jamesi													
A. karwari													
A. annularis													
A. philippinensis													

A. hyrcanus var. *sinensis* is the commonest species found throughout all the districts visited. It is interesting to note that 14 species and varieties of *Anopheles* were found in Mangshih in the low lying plains.

With regard to the breeding places of anopheles, it is difficult to give a systematic description. Each species more or less shows its own preferential breeding habits. Generally speaking, there are essentially four classes of breeding places, (1) collections of stagnant water, (2) slow or fast moving water, (3) small collections of water, (4) rice fields. These breeding places may be

sunny or shady, clear or muddy, and with or without the larger types of aquatic vegetation. Each class may be further subdivided as follows:—

(1) *Stagnant water.*—Lakes, ponds, grassy pools, stagnant ditches, fresh water swamp, wells and cisterns.

(2) *Slow or fast moving water.*—Creeks, streams, river margins, flowing ditches and springs.

(3) *Small collections of water.*—Tree holes, cut bamboo stumps, rock holes and temporary rain puddles.

(4) *Rice fields.*—The fields may be cultivated or fallow. Water may be stagnant or with a sluggish current as in terraced fields.

Relation of Malaria to Species of Anopheline Mosquitoes Found.

We base the analysis given on the results of a series of 1,387 mosquito-dissections made at Mangshih during the period July to October, 1939. These four months of the year cover the time when mosquitoes are most abundant, coinciding with the rainy season.

The anophelines studied by dissection were obtained from three main sources in Mangshih.

(1) A bedroom in the hospital attached to the South-Western Transportation Bureau.

(2) The sleeping quarters used by the drivers of the Transportation system and the Highway repair staff.

(3) Native Shan houses occupied by known malaria-infected families.

(1) The bedroom in the hospital used by my technical staff had whitewashed walls. The windows and doors were screened with cloth gauze, so in order to capture the mosquitoes which sought entrance, the doors and windows were left open during the night and closed before dawn. The mosquitoes were readily captured on the screens in the early morning. We collected as many as 132 anophelines in one morning from the screen over the window.

(2) The drivers' and Highway coolies' sleeping quarters are constructed of simple bamboo walls with a thatched roof. These bamboo walls have many crevices by which mosquitoes readily get access. The beds are fitted with mosquito nets of a kind, but they are not efficient. Two types are common, the old fashioned Chinese square net and small round nets which only cover the head. The square nets have too large a mash to provide efficient protection against *A. minimum* and the entrances to the nets have insufficient overlap to ensure proper closure during sleep.

The small round nets do not protect the entire body and, as there is hot weather in Mangshih, blankets are frequently discarded. We usually could collect from the mosquito nets an ample supply of anophelines for dissections. The drivers and Highway coolies suffered considerably from malaria and it

was customary to find several cases of malarial fever in the sleeping quarters when we made our collections.

(3) The bedrooms of the Shan native inhabitants were small and dark and afforded an ideal resting place for mosquitoes. The local Shan people, as we have noted previously, are most conservative in their customs and resent anything in the way of scientific study. When suffering from malaria they do not avail themselves of modern medical treatment but consult native herbalists. They do not seek medical aid until they have had several attacks of malaria. Our blood examinations of such cases gave a high incidence of gametocytes.

The anophelines collected were transferred to screened glass lamp chimneys placed over painted cigarette tin lids containing moist cellulo-cotton. Absorbent cotton-wool soaked in glucose solution was placed over the screened

TABLE V.

RESULTS OF DISSECTIONS.

Species of Anopheles.	Number Dissected.	Number Positive.			Per cent. Infection.
		Midgut.	Salivary Gland.	Midgut and Salivary Gland.	
annularis	392	3	2	1	1·53
minimus	340	21	9	3	9·70
hyrcanus var. sinensis ...	309	3	4	1	2·58
jeyporiensis	108	1	2	3	5·55
culicifacies	52	2	—	—	3·84
maculatus	132	1	3	—	3·03
vagus	40	—	—	—	—
hyrcanus var. nigerrimus	11	—	—	—	—
barbirostris	3	—	—	—	—

opening at the top of the chambers as food for the mosquitoes. We kept these anophelines for at least 72 hours until engorged blood was digested and only dissected mosquitoes which were still alive. This technique presented little difficulty as the humidity and temperature of the region was well suited to mosquitoes.

A preliminary report has been made on the results of these dissections (ROBERTSON, 1940). These results are now brought up to date and analysed to demonstrate which anophelines are implicated as important malaria vectors. The tables included in this paper give the results in a summarised form. Nine different species of anophelines were represented in the series of dissections. Six species of Anopheles appear to be malaria transmitters; namely hyrcanus var. sinensis, culicifacies, minimus, jeyporiensis var. candidiensis, maculatus and annularis.

Notes on the more important malaria transmitting mosquitoes in Western Yunnan.

A. minimus. This mosquito is in our opinion definitely associated with endemic malaria in Western Yunnan.

The greatest prevalence of *A. minimus* at the end of the monsoon rains coincides with the peak of the malaria incidence.

In April and May when malaria is not so serious the adult of *A. minimus* is encountered less frequently.

A. jeyporiensis var. *candidiensis* is found to be second only to *A. minimus* as a malaria carrier in this region. We found six heavy infections out of 108 dissections.

A. maculatus is also responsible for the transmission of malaria but it is not of such importance as *A. minimus* except in the dry season.

TABLE VI.

THE RESULT OF DISSECTIONS OF ANOPHELINE MOSQUITOES CAUGHT FROM THE BEDROOMS OF A MALARIA INFECTED SHAN FAMILY.

Species of *Anopheles*	Number Dissected.	Number Infected.			
		Midgut.	Salivary Glands.	Midgut and Salivary Glands.	Total.
hyrcanus var. *sinensis* ...	16	2	2	1	5
minimus	11	5	2	1	8
jeyporiensis var. *candidiensis*	1	1	—	—	1
maculatus	3	—	1	—	1
annularis	15	3	2	1	6

We consider *A. annularis* and *A. hyrcanus* var. *sinensis* to be of about equal importance. *A. hyrcanus* var. *sinensis* is one of the common species found in human dwellings and is widely distributed throughout the whole province from high plateaux of over 6,000 feet above sea level to the lower plains of Mangshih and Southern Yunnan. Yet the infectivity of this species is not so high as other common species found in the region.

We agree that *A. hyrcanus* var. *sinensis* is definitely proved to be the most important malaria carrier in other parts of China. For instance FUNG (1937) has summarised 12,334 dissections of this species and found 0·045 per cent. of natural infection. However in Yunnan we find GASCHEN (1935) noted that despite numerous dissections of this species, none was found infected. YAO and LING (1937) dissected nine at Ning-erh and found no infections.

Eleven *A. hyrcanus* var. *sinensis* were experimentally fed upon a soldier suffering from sub-tertian malaria in Sze-mao by YAO and LING (1937): only one was found positive for oöcysts.

In our series of dissections 309 *A. hyrcanus* var. *sinensis* were examined, only eight were found with malaria parasites. Of these eight, three were gut infections with half grown oöcysts in the gut. Four were gland infections and the remaining specimen showed moderate infection of the salivary gland and four mature oöcysts of the midgut. These positive findings were from mosquitoes collected in the bed-nets of infected persons. The patients had a high gametocyte rate so the chance of finding infected mosquitoes was high.

Our contention is that *A. hyrcanus* var. *sinensis* is not one of the most important malaria vectors in Yunnan province. I think this view is shared by the American malariologists who studied this area early in 1940. This theory is of immense importance in control work, as if one could deal only with *A. minimus* breeding places by oiling, paris green or drainage (leaving the rice paddies alone) and so lower malaria incidence. a great step in advance would be made. I understand this experiment is now being made at Chefang but it will require a considerable trial period before one can be certain of the results.

It is of interest to note that out of eleven *A. minimus* dissected, eight were found to harbour malaria parasites and. except one specimen with only eleven oöcysts in the mid-gut, the oöcyst count of the rest of the infected specimens showed more than 50. In one specimen there were as many as 102 oöcysts in the mid-gut.

The infection of the salivary glands was very high.

MALARIA CONTROL.

During the progress of this survey active measures were put into operation to deal with the malaria situation. The first line of attack consisted of aiming at the protection of the Chinese immigrants to the worst malarious districts with special reference to the workers on the Transportation System. The next important task was to deal with the high incidence of malaria amongst the coolies on the road maintenance gangs. The local Shan population were at first more or less ignored though an early attempt was made to make them familiar with modern medical treatment by starting a little rural health work.

The general principles at first were necessarily of a temporary and transient nature such as would be followed by an army in the field on extended lines of communication. The work expanded month by month and is still being extended along the route as far as the resources of the Chinese authorities permit. The South Western Transportation Bureau Medical Service did most of the pioneer medical work by establishing clinics and detention hospitals at all important points on the road. The Yunnan Burma Highway Bureau medical service dealt with the road repair gangs.

Clinics were established where the road maintenance gangs were operating in large numbers. Later travelling clinics in motor vans were instituted to deal with remote parts of the road. It would serve no useful purpose to enumerate the actual numbers of clinics and medical units operating at any particular time as the medical services on the Burma Highway are developing from day to day. The main principles are, however, of interest.

The Chinese Government from the first fully appreciated the need for energetic anti-malaria work on the new highway. They, however, rightly felt this should develop along lines which would include the installation of general medical facilities. The territory opened up had no existing medical services which could be expanded, so everything had to be constructed from first principles.

Treatment of Malaria

I advised that the short quinine treatment of the League of Nations Malaria Commission (1937) be used as standard. This gave satisfactory results. Intravenous quinine was used in serious cases wih cerebral symptoms. Supplies of atebrin, plasmoquine and other synthetic preparations were available in small quantities and undoubtedly were of great value in treating relapses of sub-tertian malaria; however, the quantities used did not affect the malaria incidence as a whole. Atebrin as a prophylactic was not tried out for the labour gangs although much can be said in its favour as doses can be given weekly on pay days.

In my opinion atebrin and allied drugs are to be reserved for their valued effect in reducing relapse rates when the patients are moved away from the malarious districts. Quinine hydrochloride and bisulphate tablets were our main standby. It is of interest to record that the League of Nations sent over 7 million tablets of 5 grains (nearly 2½ tons) in 1939 for this work. One of my chief concerns was the supervision of quinine distribution and the insistence upon adequate diagnosis of malaria and the application of the full course of treatment to each case. There is a great tendency for quinine supplies to be frittered away by giving a few tablets to fever cases and then losing sight of the patients. It may seem harsh but I fully believe that this form of medical treatment will never serve any useful purpose and will in no way affect malaria in the community.

Quinine prophylaxis.—This was resorted to for the drivers of the trucks and travellers along the Highway. I think the basic theory is unsound but it seemed to be the only thing to be done till other measures of control have been evolved such as de-parasitizing the indigenous population, and anti-mosquito measures.

Anti-mosquito measures.—The most promising line is to attack the breeding places of *A. minimus* by drainage, oiling and use of paris green. This has been commenced at Chefang.

The American malariologists from the U.S.A. Health Service supervised an experimental station during the spring of this year (1940) and the work is being carried on by the Rockefeller Foundation experts. The League Unit under my supervision supplied Four Oaks sprays, anti-mosquito oil and supplies of paris green.

I do not think it is practicable to do much in the way of sub-soil drainage as the rice paddies receive running water in broad terraces until it is discharged into the river. Running water is made use of in a very practical way for local agricultural needs.

Sub-soil drainage using bamboo pipes could be applied to a limited extent around the transport depôts.

It would, however, be much better policy to choose suitable sites for the new Highway stations on healthy ground.

The present villages should be avoided. They have grown up because of agricultural considerations. New villages will soon assemble round the Highway depôts and some control of the type of housing and new construction would be very advisable. Where new hotels and hostels are being erected, these should be placed on suitable sites on dry ground and not allowed as at present to be placed in the midst of insanitary Shan villages.

The following are the main recommendations:

(1) Health education amongst the indigenous population.

(2) Attempt to de-parasitize the local Shan population in the sector from Lungling through Mangshih to the frontier.

(3) Increased medical aid and travelling clinics for the roadway workers and labour gangs.

(4) Building mosquito-proof shelters for the road repair gangs at suitable points on the Highway.

(5) Placing all new depôts of the transportation system, hotels and hostels for drivers on suitable sites away from the swamps; and screening the buildings.

(6) Anti-mosquito measures where practicable directed against *A. minimus* breeding places.

(7) Provision of mosquito nets of a suitable mesh—bearing in mind that *A. minimus* is the most dangerous vector and that the present nets are of too large a mesh.

(8) The continued improvement of the medical and hospital facilities.

(9) That the diet of the coolie gangs be studied and receive supervision. There is under-nourishment and liability to deficiency diseases. Iodine prophylaxis for endemic goitre is required.

A great opportunity awaits a wise and far-sighted policy on the part of the authorities at the present moment. It is of the utmost importance at present to supervise the construction of the new settlements and have these established in the best locality available. If expensive buildings are put up in the midst of

insanitary villages a situation will arise which will be very costly to rectify in the future. It is a profound mistake to follow as at present the domiciliary habits of a malarious and partially immune agricultural population. By following a few well tested principles of malariology it will be possible to look forward to a comparatively healthy route from China to Burma.

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HISTOPATHOLOGY OF THE KIDNEY IN CHOLERA.

BY

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In cholera the signs referable to the urinary excretion are very prominent. Suppression of urine is a constant symptom. Along with this there is the retention of the nitrogenous constituents of the blood in high amounts in a large proportion of cases. (SHORTEN, 1918; ROGERS, 1921, pp. 138-183; DHAR *et al.*, 1930; BANNERJEE, 1936, and CHATTERJEE and SARKAR, 1939.)

In contrast to the above findings, the morphological changes observed in the kidneys in cases of cholera have been found to be inconstant and comparatively inconspicuous both with the naked eye as well as with ordinary methods of examination. From the postmortem records of fifty-eight cases of cholera in the Carmichael Medical College Hospitals the gross appearance of the kidneys to the naked eye showed a marked congestion in 5 per cent., moderate congestion in 36 per cent., and a practically normal structure in 59 per cent. Thus ROGERS, *loc. cit.*, wrote, " I have carefully studied the naked eye and microscopical changes of the kidneys in fatal cholera cases for some years past and find them to be very variable. Thus in cases in the acute stage nothing beyond the congestion of the organs may be found while microscopically the renal epithelium may be quite healthy."

On the postmortem table as one incises the capsule the kidney substance bulges out. On section the whole kidney might show a congested appearance. But this congestion is more prominent in the medulla so that the hyperaemic medulla stands out somewhat well marked from the cortex where the congestion is much less visible and may be apparently absent. The relation between the cortex and medulla is not disturbed. In the kidneys of uraemic cases the congestion is all the more marked.

I. HISTOLOGICAL CHANGES.

The following study consists of an examination of the kidneys in thirteen cases of uraemia following cholera, and also in another twenty-five cholera patients in whom death was due to causes other than uraemia. The stains

used were Mallory's aniline blue stain (Lee Brown's modification), haematoxylin and eosin stain, Foote's reticular stain, Van Gieson stain and peroxidase stain.

A.—HISTOLOGICAL CHANGES IN THE KIDNEY IN CASES OF URAEMIA IN CHOLERA.

1. *General Capillary Dilatation.*

The kidneys show a dilatation and engorgement of the capillaries. This congestion is most marked in the capillaries of (*i*) the glomerular tuft and (*ii*) of the medulla where the congestion is very intense. It is peculiar that other capillaries in the cortex are little affected and this is possibly due to the great swelling and engorgement of the glomerular tuft, which prevents the flow of blood into efferent vessels of the glomeruli.

2. *Malphigian Corpuscles.*

(*a*) The glomerular tufts appear to be enlarged and have a peculiar rounded and swollen appearance. Instead of a normal tuft-like structure, they are greatly swollen and seem to fill up the whole corpuscle. As observed with Lee Brown's stain the basement membrane of the glomerulus (McGREGOR, 1929, and BELL, 1935) is markedly swollen and appears remarkably thicker. This swollen basement membrane takes the stain much less prominently than is seen normally, it is also seen to split up into minute hyaline fibrils.

The glomerular tuft may show a great congestion and dilatation of the capillaries.

As a result of the above changes the renal corpuscle seems to be more or less one solid mass, often showing lobulations ; and the potential space of the Bowman's capsule where urine collects in between the parietal and glomerular layers of the epithelium, as observed in hardened sections, is more or less obliterated : thus a filling up of the renal corpuscle occurs. This filling up is not due to the increase of cellular structures, as very few cells of inflammatory origin, *viz.*, the polymorphonuclear cells, can be seen when stained by the peroxidase stain. Nor is this brought about by a multiplication of epithelial and endothelial cells. MERTZ (1918) gives the number of nuclei in the greatest diameter of the corpuscles as 145. In our series of uraemic kidneys we did not find the nuclear count to be more than 100 except only in two kidneys.

Therefore, as seen in the usual paraffin sections, the obliteration of the capsular space is brought about by a peculiar swelling of (*a*) the glomerular basement membrane and (*b*) by the dilatation and engorgement of the capillaries. In fact, the time is too short in this acute disease to allow of any marked proliferation of the epithelial structures and formation of epithelial crescents.

(*b*) The parietal layer of the basement membrane, on which a single layer of flattened cells rests, also shows similar changes, *viz.*, a great swelling and splitting up into minute fibrils.
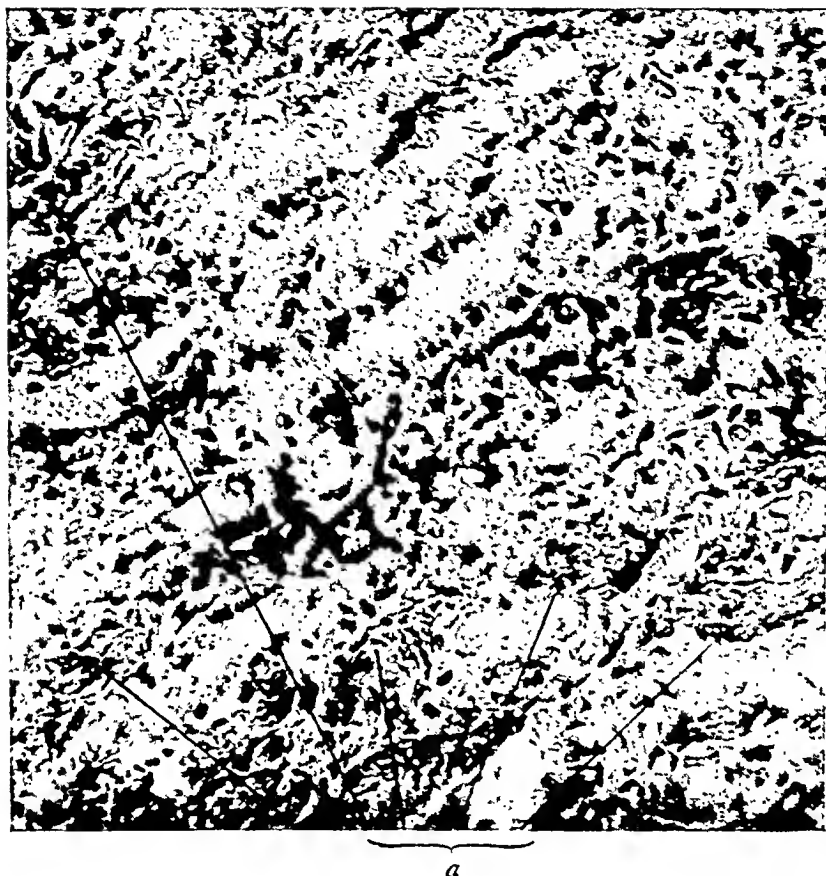
FIG. 1.—Section showing the medulla of the kidney in a case of uraemia of cholera. (*a*) the dilated and engorged capillaries.
Magnification 375 times approximately. (Haematoxylin-eosin stain.)

FIG. 3.—Section showing the cortex of the kidney in a case of uraemia of cholera. The glomeruli are swollen and greatly fill up the capsular space.
Magnification 110 times approximately. (Lee Brown stain.)

FIG. 2.—Section showing a glomerulus from the kidney of uraemia of cholera. Note the absence of proliferation of nuclei. Note also the swelling and splitting up of the basement membrane of the glomerular tuft (a).
Magnification 600 times approximately. (Lee Brown stain.)

3. *The Tubules.*

In uraemia cases there is a marked swelling of the tubular basement membrane as observed with Lee Brown's stain. In places this swollen basement membrane splits up into hyaline fibrils. There is also a marked capillary congestion. This capillary congestion is not of inflammatory nature as inflammatory cells are seldom seen.

Sometimes (as observed by us in four cases of uraemia) the cells of the tubules might show degenerative changes. But these are not constant features and might be absent in the kidneys of other cases of uraemia in cholera. The changes consist of a fatty degeneretion of the cells of the convoluted tubules (two cases) and also of a degenerative swelling of the cells so that the lumen of the tubule is obliterated (two cases).

In addition to the non-argyrophil basement membrane the writer has also observed a noticeable swelling of the argyrophil reticular network of the kidney and its disintegration by splitting up into fibrils which however still retain their argyrophil properties as observed with Foote's silver stain.

B. THE CHANGES IN KIDNEYS OF NON-URAEMIC CASES OF CHOLERA.

The changes observed in cases of non-uraemic kidneys are much less marked.

The acute congestion of the medulla and the glomerular capillaries may be seen but the other changes though present are much less noticeable and marked.

C. SOME FUNCTIONAL QUESTIONS.

From the descriptions given above it appears that acute inflammatory changes are as a rule absent in the uraemic as well as in the non-uraemic kidney of cholera.

As the changes are non-inflammatory it is comparatively easier for the kidney to come back to its normal functioning condition. Clinically this is borne out by the fact that the kidney disfunction is of comparatively temporary nature. Permanent damage to the kidney is extremely rare and it is seldom that one comes across cases of subsequent kidney failure in those cases of cholera with or without uraemia.

ROGERS (1921, p. 81) found that there is much greater difficulty in perfusing through the cholera kidney than through the normal organ. It is possible that the structural changes in the filtering membrane of the glomerulus, *i.e.* the basement membrane, produce a great difficulty in the process of filtration. This would be all the more so in the case of the nitrogenous constituents of the blood of high molecular weight. Consequently the changes described in this paper may be contributory causes of a considerable retention of the nitrogenous products in addition to the physiochemical changes in the blood mentioned

in another paper (CHATTERJEE, 1939a). It is not rare to find cases of uraemia passing urine of a low specific gravity, but showing a high nitrogenous retention. Such a case is reported below.

CASE.

P., Bengalee female, aged 23 years, cholera 14 days, presence of agglutinable vibrios from stools on admission ; reluctant to answer questions ; eyes congested ; respiration 42 per minute ; pulse 130 per minute.

Blood examination on 18.6.38.

	mg. per 100 c.c.
Urea N. ...	98·0
Uric acid	10·0
Creatinin...	5·6
Chloride ...	370·0
Cholesterol	120·0

Urine 24 ounces, specific gravity 1010.

Gradually the daily output of urine improved as also the mental condition. Patient ultimately recovered and left the hospital after a month.

It is possible that in cases such as this, a certain amount of water is allowed to pass but not the nitrogenous constituents of heavier molecular weight ; the low specific gravity of the urine can also be explained by the functional disturbance of the tubules and their inability to concentrate urine and absorb water owing to great thickening of the basement membrane as well as the engorgement of capillaries.

D. AGE INCIDENCE AND PRE-EXISTING KIDNEY DISEASE AND URAEMIA.

Pre-existing kidney disease may be thought to be responsible as being one of the causes of uraemia in cholera. The age incidence, however, shows that the majority of cases (78 per cent.) occur below the age of 35 ; this will be observed from Table I.

In fact, we found pre-existing fibrotic kidney changes in only two of thirteen kidneys examined by us, the ages of both cases being over 35.

TABLE I.

SHOWING INCIDENCE OF URAEMIA AND AGE (THIRTEEN CASES OF URAEMIA IN 3 YEARS.)

Age ...	7–10	11–20	21–30	31–35	36–40	41–60
Percentage of cases ...	11 per cent.	11 per cent.	45 per cent.	11 per cent.	11 per cent.	11 per cent.

78 per cent.

SUMMARY.

1. Histological changes found in the kidney in uraemia of cholera are described and compared with those found in non-uraemic cases.

2. The changes are of non-inflammatory and non-proliferative type.

3. The Malphigian corpuscles show a great thickening and splitting up of the basement membrane as well as a non-inflammatory dilatation and congestion of the capillaries of the glomerular tuft. As a result, the Bowman's capsule is seen to be more or less completely filled up.

4. Similar swelling of the basement membrane of the tubules and dilatation of the capillaries of the medulla are also observed.

5. Some functional aspects as well as the negative rôle of age incidence and previous kidney disease are discussed.

II. Pathological Physiology of the Kidney in Cholera.

The retention of nitrogenous products in blood is a well known fact in cholera (SHORTEN, 1918; ROGERS, 1921, pp. 138-183; DHAR *et al.*, 1930; BANNERJEE, 1936, and CHATTERJEE and SARKAR, 1939). The same nitrogenous retention and increase in the blood cannot be explained by the mere concentration of the blood. This will be proved by the fact that it is observed in those cases in which the specific gravity has been restored by the transfusion of saline and is apparent from Table II.

TABLE II.

Showing the Relation between the Specific Gravity of Blood and Nitrogenous Retention.

Case.	Date.	Clinical Notes.	Specific Gravity of Blood.	Blood urea mg. per cent.	Blood N.P.N. mg. per cent.
90H	29-5-39	Before administration of saline	1068	35·0	44·8
	31-5-39	After usual saline treatment	1056	28·0	44·8
91	29-5-39	After administration of saline	1056	21·0	42·0
	31-5-39	After continuance of saline	1054	24·5	50·4

This retention occurs in the majority of cases of cholera and may not be associated with any visible kidney changes. Thus, the non-protein nitrogen was found increased in 79 per cent. and blood urea in 72 per cent. of our cholera cases.

A high nitrogenous retention has been observed by various workers in shock and allied conditions. Thus DUVAL and GRIGAUT (1918) observed a high non-protein nitrogen of the blood in wounded soldiers. The urea nitrogen

was however only slightly increased in their cases. AUB and WU (1920) also noted similar changes in experimental shock and observed that the same was proportional to the reduction of the metabolic rate. MARRIOTT (1923) similarly observed an increase of blood urea and non-protein nitrogen in experimental shock and after injections of histamine and proteoses. UNDERHILL and his associates (1923) studied the blood changes in cases of serious burns. They found that the non-protein nitrogen, creatinin and urea were above normal in their cases. As the nitrogenous retention also occurs even in non-uraemic cases where the kidney changes are very little apparent the condition of shock may be one of the contributory factors.

The condition in which there is retention of nitrogenous bodies in the organism has been named by WIDAL and JAVAL (1905) as " azotaemia." FISHBERG (1939) defines the condition of nitrogenous retention with little or no structural alteration of the kidney as " pre-renal azotaemia."

A good deal of light has been thrown on the mechanism of this pre-renal azotaemia by experimental findings of different workers. A few of these are mentioned below.

(a) *Chloride Loss and Azotaemia.*—The work of BLUM and his co-workers has indicated a close relation between the loss of chloride and azotaemia. BLUM, GRABAR and VAN CAULAERT (1929) arrived at this conclusion by experimental investigation in diabetic patients. They found that the urea content of the blood ascended as the chloride concentration fell. Administration of sodium chloride resulted in the prompt and rapid fall in azotaemia as the hypochloraemia was diminished. That there is a great loss of chlorides in cholera is well known (CHATTERJEE, 1939a ; VAN SLYKE et al., 1934). Consequently it would not be improper to associate azotaemia with hypochloraemia.

(b) *Rôle of Decreased Renal Blood Flow in Pre-renal Azotaemia.*—In the numerous conditions that produce shock there is peripheral circulatory failure. There is also azotaemia in these cases. That there is a very close relation between these two phenomena is suggested by the work of VAN SLYKE and his co-workers. They have shown by their experiment on unanaesthetised dogs that urea clearance closely parallels the blood flow through the kidneys. FISHBERG (1939) considers this decreased blood flow to the kidneys to be the primary pathogenic factor, in most instances of pre-renal azotaemia.

(c) *Question of Histamine-like Substances.*—The great congestion of the capillaries especially of the glomerular tuft and medulla without however any inflammatory signs suggests the action of some histamine-like substance.

Similar capillary dilatation and engorgement in other organs have been described by the writer (CHATTERJEE, 1939, b and c).

BJERING (1937) has demonstrated the action of the histamine in the renal blood vessels and the reduction of urea and creatinin clearance tests that follow the injections of histamine.

HUSFELDT and BJERING (1937) in a further note describe two cases of failure

following traumatic injuries (kidney injury excluded), the renal findings suggesting histamine shock.

MARRIOTT *loc. cit.*, also observed high blood urea and non-protein nitrogen after experimental infection of histamine.

CHATTERJEE (1939c) has also shown that the cholera vibrios can produce histamine in synthetic media.

An evaluation of all the above facts mentioned so far, in relation to cholera may not be out of place here. Kidney failure in cholera is only a temporary condition. Subsequent renal failure after recovery from cholera is an extremely rare condition. Consequently it is very likely that in the average case of cholera this nitrogenous retention is due rather to extra-renal causes mentioned above than to the structural changes in the kidneys which might ultimately lead to permanent renal changes.

Suppression of Urine.

Regarding anuria which occurs in the majority of cases of cholera, it has been shown by ROGERS that this is due to deficient blood pressure, a very important cause of which is loss of fluid. Haemoconcentration has also its probable part. The glomerular capillaries are unable to produce a normal volume of filtrate from the abnormal concentrated blood (MOON, 1938.) Consequently after restoration of blood volume and lowering of the specific gravity of blood there is again the free secretion of urine, in the uncomplicated cases of cholera.

THE PATHOGENESIS OF URAEMIA IN CHOLERA.

The histological change in the kidney of uraemic cases in cholera has already been described. ROGERS gives the incidence of uraemia in his cholera cases as 13·2 per cent. A few of the associated facts about the uraemia in cholera may be discussed here.

A. *Presence of Cholera Vibrios in Urine.*

Although GREIG (1913 and 1919) was able to cultivate the cholera vibrios in only eight out of fifty-five cases, owing to the absence of complete details in his procedure, chances of contamination cannot be ruled out. CHATTERJEE (D. N.) and MALIK (1928) were however unable to get any positive vibrio cultures in 122 cholera patients in whom the specimens were collected by means of sterile catheter under aseptic conditions. GREIG, *loc. cit.*, DE MONTE and GUPTA (1938) obtained sterile blood cultures from cholera patients.

In the League of Nations Report on the *Studies of Cholera in Japan* (TAKANO *et al.*, 1926) we also find the following statement " Cholera vibrios have never been demonstrated in the blood or urine." Consequently the actual presence of the cholera vibrio in the kidneys as a factor in the production of kidney symptoms may be ruled out.

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A CASE OF MISCARRIAGE FOLLOWING BLACKWATER FEVER.

BY

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AND

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During the course of our investigation on blackwater fever in Macedonia, we had admitted to our wards a 7-months pregnant woman suffering from blackwater fever.*

Previous cases of blackwater fever during pregnancy have been reported by STEPHENS (1937), THOMSON (1924) and THOMAS and MILLEN (1939), but in none of these cases were the babies available for necropsy nor was the placental blood examined for pigments or parasites. We are therefore reporting the following case as it throws some light on the non-passage of methaemalbumin through the placenta, and the complete absence of any sign of haemolysis in the baby.

The patient, a woman of 31 years, 7 months pregnant, stated that she was " feeling out of sorts " on the morning of 5th November and went to see her doctor who then, at 9 a.m., gave her a 1 gramme intramuscular injection of quinine bihydrochloride. At midday on 6th November—29 hours after the quinine injection—she first passed black urine and continued to do so throughout the day. On 7th November she entered the hospital and was then intensely icteric, nervous, vomiting, and obviously very ill. Her spleen was III (Hackett), liver palpable and tender, pulse rapid, and skin clammy. Her temperature was 37·8° C. She gave no previous history of blackwater fever either personal or in her family.

A specimen of urine catheterized on her entrance to hospital contained 130 mg. per cent. haemoglobin, and 150 mg. per cent. methaemoglobin and had

a pH of 5·8 taken electrometrically. Numerous granular casts, kidney and pus cells were found in the urine. There were no R.B.C.s in the urine. Vein blood taken into heparin was as follows :—

R.B.C., 2,450,000
Hb : (Darc) 7·8 grammes per cent. ;
  (Newcomer) 8·1 grammes per cent.
Colour Index, 1·1
Haematocrit, 24 per cent.
Mean Corp. Volume, 100 μ^3
W.B.C., 3,960

Alkali reserve 51·6 c.c. CO_2
pH (electrometric), 7·55
OxyHb in Serum, 40 mg. per cent.
Methaemalbumin, 1 in 2 dilution
Parasites, *P. falciparum* rings
Bilirubin $\begin{cases} \text{Direct.} \quad ++ \\ \text{Indirect, } 4\cdot2 \text{ mg. per cent.} \end{cases}$

During 7th and 8th November she continued to pass black or red urine containing haemoglobin and methaemoglobin, all specimens having an acid reaction. The last specimen passed at 9 p.m. on 8th November contained 104 mg. per cent. of haemoglobin and 94 mg. per cent. of methaemoglobin and had a pH of 5·9.

At 1 a.m. midnight on 9th November (4 hours after the last urine examination) the woman gave birth to a 7-months' baby, which died about 3 hours after birth.

The dead child together with the placenta were examined in the laboratory at 9 a.m. on 9th November.

The placenta was intact and appeared normal. Blood was drawn from it and centrifuged. The serum was reddish brown, and on spectroscopic examination was found to contain haemoglobin and methaemalbumin. It was loaded with *falciparum* schizonts.

The child had been dead for about 5 hours when it was examined, and it appeared to be a normal well-formed 7-months' male child. There was no icteric tinge to the skin or mucous membranes. With difficulty blood was drawn and centrifuged. The serum so obtained was clear to naked eye examination, but spectroscopically contained a faint band of haemoglobin in a cell 5 cm. thick. This was, no doubt, traumatic. There was no trace whatever of a band in the red region of the spectrum in a cell 15 cm. thick, so that methaemalbumin could be definitely ruled out. There was no sign of either malaria parasites or pigment in the infant's blood or spleen.

At 11 a.m. on 9th November a sample of vein blood was taken from the mother. The red blood cell count had dropped to 2,010,000. There were 56 mg. per cent. of haemoglobin in the serum and methaemalbumin in a concentration of 1 in 3. Indirect bilirubin, 3·8 mg. per cent. There were no parasites, but traces of malaria pigment were found here and there in the thick film.

Urine cathetered on the 9th contained traces of haemoglobin and methaemoglobin. Thereafter, the urine cleared and the woman made an uneventful recovery without any blood transfusion. She was, however, later removed to the Psychiatrion.

Comment.

The case is of interest in that the baby and the placenta were available for postmortem examination; and also that a fairly complete laboratory examination of the woman was made both before and after the birth of the child.

From an examination of the placental blood it seems that methaemalbumin does not pass from the mother across the placenta to the child. It has already been shown that this pigment does not normally pass across the kidney to be excreted in the urine,* the only pigments present in the urine in blackwater fever being oxyhaemoglobin and methaemoglobin.

Further, although the mother was undergoing haemolysis at the time the child was born (as shown by blood and urine examinations immediately before and after birth) there was no trace of this haemolytic process in the serum of the child, which contained neither haemoglobin nor methaemalbumin; nor was there any icteroid tinge of the skin or mucous membranes of the child.

If the haemolysis of the mother was due to circulating haemolysins then these never penetrated the placenta to reach the offspring and produce haemolysis there. That the products of haemolysis were removed from the child is possible but not likely.

It is not impossible that the red cells of the mother were more liable to haemolysis for some unknown reason, than those of the infant. Or, alternatively, the cells of the mother and child might have been equally liable to haemolysis when exposed to certain " unknown factors," but that these " unknown factors " did not pass the placenta and affect the red cells of the infant.

It is also possible that all the haemolysin available was used up in producing the haemolysis in the mother, and that none was left over to bring about a haemolytic process in the child. This argument, that all the haemolysin is used up, may explain why attempts to demonstrate the presence of haemolysins in cases of blackwater fever have, so far, failed.

The lack of parasites in the child may also be a factor in the absence of haemolysis in the child's blood. If the disintegration of malaria parasites can produce blackwater fever by the liberation of toxins, then the absence of parasites from the child might account for the absence of any sign of haemolysis, always supposing that the so-called toxins could not pass the placenta.

Any, or all, of these explanations may fit the case, but until more evidence is available, no good purpose can be served by theorising. Cases of blackwater fever in pregnant women, where both the placenta and child are available for laboratory examination, are all too rare. Should they occur, we think that examinations on the lines we have indicated here may throw some light on the problem of blackwater fever.

* It has been recently shown (FAIRLEY, 1939) that methaemalbumin is dealt with by the liver, as is bilirubin, and never appears in the urine.

SUMMARY.

1. A case of blackwater fever in a pregnant woman who gave birth to a 7-months' child, which died shortly after birth and was available, together with the placenta, for postmortem examination.

2. The placenta was found to be loaded with *P. falciparum* parasites, and the serum contained both haemoglobin and methaemalbumin.

3. The baby had no parasites in the blood or spleen; nor was methaemalbumin present in the serum. There were traces of traumatic haemoglobin in the serum. There was no icterus visible in the child's skin or mucous membranes.

4. The mother's blood, examined after the birth, contained both haemoglobin and methaemalbumin; and the urine haemoglobin and methaemoglobin.

5. It seems from this case that methaemalbumin does not pass over the placenta to the child.

The absence of any sign of haemolysis in the child may be due to the fact :—

(*a*) That any haemolysins circulating in the mother did not pass through the placenta to the child.

(*b*) That any haemolysin present in the mother was only there in sufficient concentration to bring about haemolytic process in the mother, and none was available to produce a haemolytic process in the child.

(*c*) Assuming that malaria parasites, or their metabolic products, are necessary for the production of blackwater fever, then the absence of all traces of parasites and malaria pigment from the baby may account for the absence of all signs of haemolysis in the child. Further, sensitization of the mother did not appear to affect the child.

(*d*) That the cells of the mother were more liable to haemolysis than those of the child; or had been sensitised by parasites or their metabolic products.

Any or all of these possibilities may account for the absence of signs of haemolysis in the child; until more evidence is available, speculation is not likely to be of much value.

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CORRIGENDUM.

Vol. 34, No. 2, August, 1940.

FAIRLEY, N. HAMILTON. A peculiar haemolytic hypochromic anaemia.

Page 179, line 8 :

or " sclerotic " *read* " siderotic."

COMMUNICATIONS.*

THE TREATMENT OF SPRUE WITH VITAMIN B$_2$ AND ITS BEARING UPON THE AETIOLOGY OF THIS DISEASE.

BY

PHILIP MANSON-BAHR, C.M.G., D.S.O., M.D., F.R.C.P.

For many years the writer has paid a good deal of attention to the aetiology of sprue and its treatment. For various reasons already stated (MANSON-BAHR, 1940) he is convinced that in this disease, as in pellagra, there exists a pre-sprue or larval condition characterized by glossitis which may precede by months the full development of the sprue syndrome, or even as long as a year ; and further-more, that no specific changes can be distinguished of sufficient importance to differentiate this glossitis from that of larval- or pre-pellagra. A somewhat parallel sequence is also to be observed in the glossitis of pernicious anaemia as well as in that of idiopathic steatorrhoea, diseases which possess features in common with sprue. It therefore appeared probable that the glossitis is non-specific and common to this group of allied diseases. Since the therapeutic application by SPIES (1937) of that portion of the vitamin B$_2$ complex—or the

* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

A

pellagra-preventive (PP) factor—in the form of nicotinic acid to pellagra, and its readily observable effect upon the pellagrous glossitis, it would appear likely that in this discovery we have been provided with a possible explanation of a link uniting these various syndromes. It has already been suspected on clinical grounds that there are some underlying aetiological factors in this disease group so closely dovetailed that, though some of the tenons and mortises are recognized, · still others remain to be discovered. The question, which can only be partially answered at present, seems to be whether similar clinical signs and symptoms can be regarded as indicating closely related aetiology for, if this is so, their response to similar therapeutic treatment should also in the main run parallel. It is in this direction then that the response of the glossitis and stomatitis of this group of diseases to nicotinic acid and riboflavin therapy may well provide a clue.

RECENT WORK ON THE VITAMIN B₂ COMPLEX AND OTHER FACTORS CONCERNED.

The possible existence of a pellagra-preventive vitamin was first postulated by FUNK (1912), whilst the experimental production of the pellagrous syndrome in man was effected by GOLDBERGER *et al.* (1926). Nicotinic acid has long been known. It was first synthesized by ENGLER in 1894 and FUNK (1912) isolated it from yeast and rice ; WARBURG and CHRISTIAN (1935) obtained it from heart ·muscle ; and ELVEHJEM (1937) nicotamide from liver. Nicotinic acid, an oxidation product of nicotine, is pyridine B-carboxylic acid and is widely distributed in liver, yeast, milk, cheese and eggs in association with other vitamins of the B complex ; thus 1 kg. of dried yeast contains 625 mg. of nicotinic acid. It is thought that nicotinic acid is either the PP (pellagra-preventive) factor, or is a provitamin. Nicotamide, a constituent of |the coenzyme—co-enzymase, which is thought to play an important rôle in carbohydrate metabolism, is the amide of nicotinic acid and is closely related to coramine, a preparation which has long been known as a cardiac stimulant and which is the diethylamide of nicotinic acid.

Subsequent to the work of GOLDBERGER on the experimental production of pellagra in man, black-tongue* disease, which is analogous with human pellagra, was produced in dogs. This disease has been reproduced in monkeys by L. J. HARRIS (1937 and 1939), and in pigs by BIRCH, CHICK and MARTIN (1937) and CHICK *et al.* in 1938. BIRCH, GYÖRGYI and HARRIS (1935) described the PP factor as a third component of the vitamin B₂ complex, but state that it differs from the rat-pellagra factor and from riboflavin : it was soon proved to be identical with nicotinic acid.

* Formerly known as " canine typhus," black-tongue occurs commonly in various parts of the United States. It is opportune to note that it produces ulceration and a thickened, swollen, red, or bluish-coloured tongue. The disease causes a mortality of 50-75 per cent. in those attacked, especially young animals, whilst the efficacy of raw meat treatment has long been recognised. (BRUMLEY, O. V., 1931, *Diseases of the Small Domestic Animals.* 2nd ed. 557.)

In 1937 ELVEHJEM tested the effect of nicotinic acid upon dogs with black-tongue and very soon afterwards it was tried by SPIES (1937) on human pellagra with obvious and now widely recognized success.

That nicotinic acid is a necessary constituent of the medium for the growth of bacteria was foreshadowed by HUGHES (1932), who found a substance capable of stimulating growth of staphylococci in meat extracts dialysed through collodion membranes and which was rapidly destroyed in alkaline solution. KNIGHT and FILDES (1933) soon after found that this essential was nicotinic acid in the case of *Staphylococcus aureus*, and MUELLER showed that it was essential for the growth of the diphtheria bacillus.

THE LWOFFS have shown that bacilli of the influenza group, which require such a coenzyme for growth, are unable to synthesize it from nicotamide, and VILTER and his colleagues (1939) have demonstrated that the blood of advanced pellagrins with a low coenzyme content cannot support the growth of this bacillus, whilst KOSER and his co-workers demonstrated that this coenzyme is a growth-promoting factor for various strains of dysentery bacilli ; FILDES that it is essential for the growth of *B. proteus*.

SPIES, BEAN and ASHE (1939) have demonstrated that nicotinic acid and nicotamide, not only reduce the fiery red glossitis, but also exterminate the " Vincent's infection " which is invariably associated with these lesions. In black-tongue in dogs MILLER and RHOADS (1934 and 1935) have also found that the associated spirochaetes and fusiform bacilli disappear on the administration of nicotinic acid, and KING (1940) finds that the infective gingivitis correlated with these organisms is influenced in the same manner.

The estimation of nicotinic acid in the blood by chemical methods has so far presented many difficulties ; but by using the observation of FILDES, already recorded, that the only growth factor required by *Proteus* was nicotinic acid, or nicotamide, QUERIDO, LWOFF and LASTASTE (1939) have worked out a method for estimating this substance in human blood. BALLIF *et al.* (1939) made estimations of the blood nicotamide in ten pellagrins. These cases all showed characteristic skin lesions, one glossitis and two achlorhydria, and in all normal values were found for nicotamide. They suggest, as an explanation of the low values that others have found, loss by the diarrhoea which so often accompanies pellagra. But whatever may be the true facts about the nicotamide-content of the blood in pellagra, there can be few doubts about the therapeutic value of its administration.

It is necessary to state that some authorities, notably STANNUS (1940), are not entirely satisfied with the finality of the idea that nicotinic acid is the true PP factor, but believe that in pellagra there is a faulty production of coenzyme (codehydrogenase).

The two coenzymes which are of immediate interest are known as coenzymes I and II. These are composed of adenine (a purine allied to guanine, xanthin, and uric acid) and nicotamide, each united to a molecule of pentose. The chemistry of these connecting

links is so involved as to be intelligible only to a specialist in this department, but any break in the link of these delicately balanced substances may initiate the train of processes leading to pellagra.

It is therefore interesting to note that VILTER and SPIES (1939) have now found that quinolinic acid, the 2·3-dicarboxylic acid of pyridine (which is non-effective in canine black-tongue) cures the acute glossitis of pellagra within 24 hours and the concentration of coenzymes I and II in the blood rises to normal.

BILLS, McDONALD and SPIES (1939) have also found that pyrazine-2, 3-dicarboxylic acid, the homologue of quinolinic acid—(which is not known to occur in any physiological substance, or in any known vitamin) in doses of 100 mg. five times daily also cures pellagrous glossitis. Pyrazine mono-carboxylic acid was found to yield the same response, whilst both substances produce an increase in coenzymes I and II in the blood and urine.

VITAMIN B₂ (LACTOFLAVIN, RIBOFLAVIN, VITAMIN G).

It was found that the antineuritic factor of vitamin B was destroyed by heat, leaving a growth-promoting substance, which was named B₂ (or G in U.S.A.). A new oxidation enzyme was obtained from yeast and described by WARBURG and CHRISTIAN in 1932. The identity of vitamin B₂ with the yellow pigment present in WARBURG's enzyme with the yellow-green fluorescent pigments in living tissues was established by KUHN in 1933, and was later named lactoflavin, or riboflavin. It has been isolated from milk, eggs and liver and synthesized by KARRER in 1935. Riboflavin is 6 : 7-dimethyl-9-[d-1-ribityl]-isoalloxazine. 1 kg. of yeast contains 20 mg. of riboflavin which is a yellow-green fluorescent water-soluble pigment widely distributed throughout the plant and animal kingdom. Riboflavin forms a phosphoric acid ester which combines with a protein to yield a yellow oxidation enzyme and is responsible for the greenish-yellow colour of whey. The human requirements are about 2 to 3 mg. daily. This amount is necessary for growth, is essential for cell-respiration, and in the organism it acts in combination with phosphoric acid. The therapeutic indications are for retarded growth in children, for coeliac disease, angular stomatitis (cheilosis) and anaemia. The part it may play in pellagra is shown by its influence on the mouth lesions. A deficiency of riboflavin has been found to produce *cheilosis* (angular stomatitis) which has been experimentally produced in man by SEBRELL and BUTLER (1937–1939).

These observers succeeded in producing angular stomatitis, together with seborrhoeic accumulations at the naso-labial folds, in thirteen out of eighteen women fed on a special low riboflavin dietary. This condition they suggest should be termed " ariboflavinosis." These lesions yielded to riboflavin therapy, but were uninfluenced by nicotinic acid. The dosage necessary to produce therapeutic effects varies from 5-50 mg. per day (SPIES, BEAN and ASHE, 1939). When the riboflavin was discontinued the lesions reappeared, but were again cured by riboflavin therapy.

JOLLIFFE *et al.* (1939) have found that seborrhoeic excrescences in the naso-labial folds, on the alae nasi, and on the bridge of the nose and forehead are rapidly cured by administration of riboflavin, but showed no response to diets deficient in the vitamin B complex, or to nicotinic acid. As part of the riboflavin deficiency syndrome it is to be noted that the epithelium of the lips,

especially the lower, shows degenerative changes. STANNUS has very sensibly pointed out that the particular areas of the skin involved in early pellagrous lesions—the angles of the mouth, palpebral fissures, lips, prepuce, anus, vulva and scrotum—are of very fine texture and present certain common histological features and they are, moreover, sites subjected to repeated trauma, are warm and moist and these factors determine their special liability to become affected. It is more than probable that the partial or complete picture of pellagra is produced accordingly to the presence of faults in the links of the katalytic chain.

A third component of the vitamin B_2 complex, known as vitamin B_6 (adermin, or rat-pellagra factor) has been isolated. Riboflavin is closely allied to it and is an oxazine derivative of it.

The whole subject is very complex and this is shown by the fact that the *extrinsic factor* of STRAUSS and CASTLE (1932) is a component of the PA factor in pernicious anaemia and sprue. It has, however, an individuality distinct from riboflavin, vitamin B_6 and the PP factor (BIRCH, GYÖRGI and HARRIS, 1935).

THE TREATMENT OF PELLAGRA AND ALLIED CONDITIONS WITH VITAMIN B_2.

As already noted, ELVEHJEM in 1937 tested the effect of nicotinic acid on dogs with black-tongue. The results were so satisfactory that it was subsequently applied to human pellagra by SPIES in 1937 and the results have been summarized by SPIES, BEAN and ASHE (1939).

They have controlled these observations by examination of the tongue and mouth as well as by gastroscopic examination in pellagrous patients (LEON SCHIFF and R. STEVENS), and have noted that the diseased mucous membranes of the stomach are similar in appearance to those of the oral cavity. The administration of adequate amounts of nicotinic acid, or one of its compounds, is followed by the disappearance of many symptoms of the disease within 24 to 72 hours and the fiery redness and swelling of the tongue, gums, throat and vagina subside, whilst the associated Vincent's infection disappears. But perhaps the most dramatic of all is the subsidence of acute mental symptoms.

The dose of nicotinic acid and its compounds required for any given pellagrin appears to be variable, but 500 mg. a day in divided doses is usually effective. It has been noted by a number of investigators that the administration of large amounts of nicotinic acid is followed by a sensation of heat and tingling of the skin. This is accompanied by a rise in skin temperature, especially that of the face ; and in certain individuals an erythematous rash may appear, as has been noted by the author. It would appear that nicotinic acid is necessary for the normal functioning of the gastrointestinal tract, the skin and nervous systems.

In "alcoholic" or secondary pellagra nicotinic acid does not prevent, retard, or relieve the symptoms of peripheral nerve involvement, but it does cure the glossitis and effectually heals the buccal ulcers which are such a feature of this disease in dogs.

ELVEHJEM, MADDEN, STRONG and WOOLLEY (1938) have effectively demonstrated that the activity of liver, long recognized by veterinarians as effective in the treatment of black-tongue in dogs, owes its curative value to the amount of nicotinic acid and nicotamide which it contains. But whether much of the nicotamide occurs in liver as nicotamide itself or whether it occurs in a more complex form cannot be answered at present. In any case, possibly the activity of the extrinsic factor in liver in pernicious anaemia and in allied diseases may be correlated with its potential supply of nicotinic acid and its amide.

A single dose of liver extract equivalent to 200 grammes of fresh liver fed to a dog suffering from black-tongue cures the symptoms and gives a continual growth response for one week; and a single dose of 50 grammes of nicotinic acid amide gives a similar result. It is, therefore, concluded that 100 grammes of fresh liver contains about 25 mg. potential nicotinic acid amide.

EXTRINSIC AND INTRINSIC FACTORS.

In order that transformation of the megaloblast into the mature normoblast may take place, it is necessary to supply the bone marrow with the haematinic, or PA factor, which is probably stored in the liver and which is in turn produced by the interaction of an intrinsic factor present in normal gastric juice with an extrinsic factor. This maturation cannot be effected unless the intrinsic factor is present in the gastric juice. The extrinsic factor is, on the other hand, supplied in the food taken in, so that the resulting haematinic factor is absorbed. STRAUSS and CASTLE are of the opinion that the extrinsic factor is closely related to vitamin B₂, if not that vitamin itself. Now, however, according to L. J. HARRIS, though the extrinsic factor has a distribution and stability to heat resembling that of the vitamin B₂ complex, it is distinct from the riboflavin or the PP factor (nicotinic acid).

The evidence so far obtained is that this extrinsic factor is present in yeast preparations, such as marmite, which are efficient in the megalocytic hyperchromic anaemias of tropical sprue and idiopathic steatorrhoea, but which are ineffective in pernicious anaemia. It has therefore been suggested that anaemias reacting to marmite and yeast preparations are due to lack of extrinsic factor. In this connection MILLER and RHOADS have approached the problem from another angle and have succeeded in producing in pigs a disease which resembles pernicious anaemia, characterised by megalocytic anaemia, lesions of the buccal mucosa, achlorhydria and loss of haemopoietic activity of the gastric juice, on a diet which produces black-tongue (or pellagra) in dogs and were able to cure them at a later date by oral and parenteral administration of liver extract. They concluded that the evidence for the identity of vitamin B₂ (G) and the antipernicious anaemia extrinsic factor is unsatisfactory. So far the complete symptom-complex—including glossitis, macrocytic anaemia and achlorhydria—

have not been produced as a complete syndrome, as in pernicious anaemia, in any species of animal lower than man.

The intrinsic factor of CASTLE, which is contained in the normal gastric secretion of man, is generally thought to be of the nature of an enzyme, and MEULENGRACHT (1935) has shown comparatively recently that in the pig's stomach the intrinsic factor is secreted by the pyloric glands of the stomach and the Brunner glands of the duodenum.

THE RESPONSE OF GLOSSITIS AND STOMATITIS OF VITAMIN B_2 DEFICIENCY TO NICOTINIC ACID.

Pellagrous glossitis and stomatitis has been described many times. In 1920 J. I. ENRIGHT (with whom the writer was associated) was able to observe the gradual unfolding of the pellagra syndrome in German and Austrian prisoners of war. The appearance of the tongue with marginal excoriations at the angles of the mouth (angular stomatitis) exactly resembled that figured by SPIES, BEAN and STONE (1938). These observers noted this condition in the typical diet-produced disease, in secondary pellagra, and especially in alcoholic cases. There are no features which could differentiate it from endemic glossitis, in the absence of other accompaniments of pellagra, which have been described by the writer in Ceylon (BAHR, 1915), by NICHOLLS and NIMALSURIYA, by others in Palestine, Sierra Leone, Kenya, Belgian Congo, West Africa, S. India, Malaya, China, Cuba and the West Indies, whilst ODEN and his colleagues have found a similar disease in the southern United States. This subject has been surveyed by C. D. WILLIAMS (1940) who considers that the term " pellagra " should be reserved for the classical condition, whilst unclassifiable dermatitis or other manifestation associated with nutritional defects should be termed " pellagroid."

The writer would like to make it clear that it is a stomatitis with angular lesions which is amenable to nicotinic acid therapy, but that it does not check *all* forms of stomatitis.

It is probable that this form of glossitis is identical with the state known as " kwashiorkor " described by WILLIAMS in breast-fed children in W. Africa and in similar cases with oedema in Kenya by GILLAN and in Uganda by TROWELL. The occurrence of oedema in association with typical endemic pellagra is by no means uncommon as demonstrated by BIGLAND (1920) in his Turkish prisoners of war.

It has already been pointed out by KATZENELLENBOGEN in Palestine and by AYKROYD and his colleagues (1938 and 1939) in India that this nutritional glossitis and stomatitis is amenable to nicotinic and riboflavin therapy.

There are reasons for believing, as has already been stated by the writer, that pellagrous glossitis is by no means uncommon in England in patients suffering from intestinal derangements and undergoing dietetic restriction,

TABLE I

SPRUE CASES SHOWING THE RELATIONSHIP OF THE FRACTIONAL TEST MEAL TO THE ANAEMIA, BILIRUBINAEMIA, ETC.

Representative cases selected out of a series of 300 cases investigated in the period 1929-1939

(A) = Achylia. (B) = Hyperchlorhydria. (C) = Normal acid curve. (D) = Hypochlorhydria. (E) = Achlorhydria with response to histamine

Case No.	Date, Sex and Age.	Fractional Test Meal and Glossitis.	Diarrhoea and Emaciation.	Anaemia.	Van den Bergh reaction, Blood calcium and Oral Glucose Curve.	Treatment and Progress
1	F. 1936 (A) 58	Achylia gastrica persistent. Glossitis +++, with angular stomatitis	++ (Faecal fat 25 per cent.). Loss weight 28 lb.	+ R.B.C. 3,000,000, Hb. 74 per cent., Megalocytes, anisocytosis	Indirect 0·2 unit. 9·2 mg. per cent. Flat curve	HCl + liver injections (58 c.c. campolon). Also had Bacilluria. Tongue improved in 14 days. Made a satisfactory recovery. Increase weight 1¼ lb.
2	M. 1939 (A) 52	Achylia gastrica. Glossitis +++,with angular stomatitis	+++ (Faecal fat 30 per cent.). Loss weight 36 lb.	+++ R.B.C. 1,800,000, Hb. 40 per cent. Megalocytes + anisocytosis. Leucocytes 3,000	Indirect 1·0 unit, 9·0 mg. per cent. Flat curve	HCl + liver injections (60 c.c. campolon). Improvement in general condition and especially in tongue. Reticulocyte rise to 20 per cent. Made eventual satisfactory recovery and returned to Ceylon.
3	M. 1934 (B) 45	Hyperchlorhydria, HCl 0·327 per cent. with response to histamine. Glossitis ++, with angular stomatitis	++ (Faecal fat 38 per cent.)	+ R.B.C. 3,000,000, Hb. 80 per cent., Megalocytes, anisocytosis Leucocytes 6,000	Indirect 0·2 unit, 10 mg. per cent. Flat curve	High protein diet. Liver therapy (Campolon 40 c.c.). Acid dyspepsia. Satisfactory haematological response. Increase in weight 12 lb.
4	M. 1938 (B) 57	Hyperchlorhydria HCl 0·219 per cent. with response to histamine. Glossitis ++, with aphthae	+++ (Faecal fat 48 per cent.). Loss weight 28 lb.	++ R.B.C. 2,400,000, Hb. 60 per cent. Megalocytes, anisocytosis, Leucocytes 6,000.	Indirect 3 units. 9·4 mg. per cent. Flat curve	Blood transfusion. Liver diet. Alkalis. (Campolon 78 c.c.). Rapid reticylocytosis to 20 per cent. Blood returned to 3,500,000 in 16 days. Progress satisfactory. Increase of weight.

5	M., 1931 (C) 55	Normal acid curve with response to histamine, Glossitis +++, with aphthae	+++ (Faecal fat 30 per cent), Loss weight 30 lb.	+++ R.B.C. 2,420,000, Hb, 50 per cent, Megalocytes, anisocytosis	Indirect 0·2 unit, 11 mg. per cent, Flat curve	Intensive liver therapy. Parenteral injections of campolon. Protein diet. Complicated by eczema. Satisfactory reticulocyte and haematological response to 4,400,000. Hb. 85 per cent.
6	M., 1931 (C) 44	Normal acid curve with response to histamine, Glossitis ++	++ (Faecal fat 35 per cent), Loss weight 28 lb.	O R.B.C. 4,000,000, Hb, 86 per cent, Leucocytes 6,400	Indirect 1 unit, 11 mg. per cent, Normal curve	Diet and liver. Diarrhoea ceased. Improved.
7	M., 1931 (D) 48	Hypochlorhydria with response to histamine, Glossitis ++	+++ (Faecal fat 26 per cent), Loss weight 20 lb.	+++ R.B.C. 1,920,000, Hb, 42 per cent, Megalocytes, anisocytosis, normoblasts Leucocytes 4,700	Indirect 2 units, 10 mg. per cent, Flat curve	Blood transfusion. Intensive liver therapy. High protein diet. Did well temporarily. Reticulocyte response up to 27 per cent. Relapsed later.
8	M., 1931 (D) 44	Hypochlorhydria with response to histamine, Glossitis +++, with aphthous stomatitis	++ (Faecal fat 41 per cent), Loss weight 21 lb.	++ R.B.C. 2,300,000, Hb, 50 per cent, Megalocytes, anisocytosis, Leucocytes 4,000	Indirect 4 units, 10·1 mg. per cent, Flat curve	High protein diet and parenteral liver therapy. Did well, but died of pneumonia three months later
9	M., 1930 (E) 61	Achlorhydria with slight response to histamine, Glossitis +++, with angular stomatitis	+++ (Faecal fat 23 per cent), Loss weight 28 lb.	+ R.B.C. 3,000,000, Hb, 70 per cent, Megalocytes, anisocytosis Leucocytes 6,000	Indirect 0·8 unit, 6·9 mg. per cent, Normal curve	Protein diet. Liver extract by mouth. Liver soup. HCl. Increase in weight 22 lb. in 56 days. Satisfactory haematological response. Great improvement
10	M., 1930 (E) 63	Achlorhydria with slight response to histamine, Glossitis +++, with aphthous stomatitis	++ (Faecal fat 26 per cent), Loss weight 28 lb.	+ R.B.C. 4,000,000, Hb, 80 per cent, slight megalocytosis Leucocytes 6,800	Indirect 0·2 unit, 10 mg. per cent,	Protein diet. Parenteral liver injections (Campolon 16 c.c.). HCl and nicotinic acid. Megacolon. General improvement, increase of weight, cessation of diarrhoea.

though, of course, not all cases of aphthous stomatitis to be observed in general practice are amenable to nicotinic acid therapy.

It is a matter for further enquiry whether " pellagroid " symptoms may not be discernible in what has hitherto been known as the " Plummer Vinson syndrome "—this is a condition, recognized since 1919, which is characterized by glossitis, dysphagia and anaemia in cachectic middle-aged women. According to HURST the more scientific designation is achalasia of the pharyngo-oesophageal sphincter giving rise to dysphagia—a symptom which is, moreover, by no means uncommon in pernicious anaemia, pellagra and tropical sprue.

The glossitis of pernicious anaemia closely resembles that of sprue and pellagra and, according to WILKINSON, CASTLE and MINOT (1936) soreness of the tongue, sometimes progressing to ulceration, is found in 61 per cent. of cases, a figure which approximates the 75 per cent. incidence in the writer's series of tropical sprue.

UNGLEY (1938) believes that the absence of the intrinsic factor in the gastric juice of this disease is responsible for the glossitis, abnormal haemopoiesis and associated intestinal manifestations. MIDDLETON and STEENBOCH (1933) have already found that in pernicious anaemia glossitis there was prompt response following a high vitamin B_2 intake in the absence of specific liver therapy.

Certain cases of pernicious anaemia so closely resemble tropical sprue that differential diagnosis becomes a matter of considerable difficulty. The characteristics of the associated anaemia are very similar in both diseases, and even the van den Bergh reaction gives much the same results (Table I) ; the achlorhydria and achylia gastrica, which are such cardinal features in pernicious anaemia, do not always afford an absolutely reliable index for differentiation—for achylia gastrica, hypochlorhydria and even hyperchlorhydria may not be uncommon accompaniments of tropical sprue (Table I). Acidity of the gastric juice does not appear to influence the course of the disease and therefore in sprue it cannot be correlated to the absence of the intrinsic factor. According to OLLEROS (1938) the stomach of sprue exhibits the same diffuse atrophy as that of pernicious anaemia so that it has been found impossible to correlate anatomical findings with those of experimental therapeutics as MAGNUS and UNGLEY (1938) have shown. MEULENGRACHT, in his work on the pig's stomach, in which the anti-anaemic factor is obtained from the pyloric and duodenal glands, but not from the body of the stomach, has suggested that these glands, though strictly intact, may fail in their function, whilst JONES, BENEDICT and HAMPTON (1935) claim that the stomach in pernicious anaemia may regenerate under liver therapy, just as the tongue epithelium regenerates ; the same principle may probably also be applicable to sprue. A significant feature pointed out by JACOBSON is that the anti-anaemic activity of the alimentary canal corresponds closely with the distribution of argentaffine cells : those in the cardiac region of the stomach and in fair numbers in the pylorus, but they are most numerous in the duodenum. In sprue and pernicious anaemia they are absent.

The geographical distribution of pernicious anaemia in relation to that of tropical sprue appears to be of considerable importance with reference to the possible relationship between the two diseases and is a subject to which insufficient attention appears to have been paid in the past. The fact appears to be that pernicious anaemia is extremely rare, if not entirely absent, in tropical natives, as has been stressed by DE LANGEN and LICHTENSTEIN (1936) who have asserted that in 20 years' experience in Java they have never found a case. In Egypt, AZMY et al. (1939) have referred to this subject, whilst DAVIDSON and GULLAND (1930) assert that pernicious anaemia is never seen in full-blooded negroes. The writer has searched his records for the last 20 years without being able to discover a single instance in his tropical native cases ; and in his experience true pernicious anaemia is of rare occurrence even in Europeans in the tropics. It may well be that, as in the case of sprue, Cooley's anaemia and sickle-cell anaemia, pernicious anaemia exhibits a distinct racial incidence and it is suggested that the peculiarities of this distribution may eventually shed some light upon the true aetiology of sprue. A brief reference must here be made to the glossitis of idiopathic steatorrhoea which occurs in a considerable proportion of cases (twenty-six out of thirty-four in THAYSEN's series), the appearances of which are identical with that of sprue and pernicious anaemia as indicated by HANSEN and STAA (1936). The writer's recent experiences have shown that this form of glossitis is amenable to nicotinic acid therapy, though other manifestations of the disease do not respond in the same satisfactory manner.

The collecting and summarizing of these various scattered observations have been undertaken with the object of shedding some light upon the aetiology and subsequent treatment of tropical sprue. Table II (page 358) summarizes the generally accepted points of distinction between the various clinical states which have been observed.

SPRUE REGARDED AS A DEFICIENCY DISEASE.

It has already been suggested by ELDERS (1922), NICHOLLS (1918), and MACCARRISON (1919) that sprue may be a vitamin-deficiency disease, but this view has not, up to the present, met with general acceptance, mainly because sprue usually occurs in well-to-do and apparently well-fed people who are unlikely to have been deprived of the essentials of life. In 1934 NICHOLLS, on the ground of the relationship of the pathology of sprue to pellagra and their reaction to a rich vitamin dietary, formulated a hypothesis that the genesis of these diseases is due to faulty absorption from the small intestines in which the vitamins are mainly concerned. In the main this hypothesis is in agreement with the views expressed in this paper and also with the author's previous communication in the Lancet (MANSON-BAHR, 1940) ; it is suggestive, too, that the conclusions of BENNETT and HARDWICK (1940) upon the clinical sprue-like syndrome (" chronic ileojejunal insufficiency ") attributable to disturbed function of the small intestine also fall into line.

TABLE II.

DIFFERENTIAL DIAGNOSIS.

Pernicious Anaemia.	Tropical Sprue.
Age. 40-60 years.	*Age.* 20-60 years.*
Distribution. Apparently absent or rare in tropical natives. Mostly in well-fed Northern Europeans.	*Distribution.* Peculiar tropical distribution—(Central Africa excluded). Mostly in Europeans and well-fed people.
Cause. Intrinsic factor absent; does not return on treatment. Preceded by achylia gastrica. Atrophic gastritis.	*Cause.* Intrinsic factor absent; returns on treatment. Hypochlorhydria. (Achylia gastrica rare). Atrophic gastritis. Previous intestinal disease predisposing factor.
Course. Onset insidious. Glossitis common, dysphagia rare. Wasting slight. Spontaneous remissions distinct feature. Diarrhoea frequent. Bilious stools. Peripheral-like polyneuritis. Changes in central nervous system common (subacute combined degeneration). No bone changes.	*Course.* Onset insidious. Glossitis common, dysphagia occasional. Wasting extreme. Remissions distinct feature. Steatorrhoea. Fatty acids; soaps scanty. Whole alimentary tract involved, especially small intestine, megacolon occasional. Changes in central nervous system very rare. Osteoporosis not observed.
Pathology. Megalocytic anaemia, and leucopenia. Megaloblastic hyperplasia of bone marrow. Bilirubinaemia. Blood glucose curve normal. Blood calcium normal.	*Pathology.* Megalocytic anaemia, and leucopenia. Bilirubinaemia occasional. Flat blood glucose curve. Hypocalcaemia. Tetany occasional. Bone marrow atrophic.
Treatment. Responds to liver fraction. Campolon—anahaemin, and to some extent to vitamin B₂.	*Treatment.* Responds to liver fraction. Campolon — anahaemin intramuscularly in large doses, + vitamin B₂.
Relapses. Relapses incvitable, unless reservoir dosage of liver extract is maintained.	*Relapses.* Spontaneous recovery frequently observed. May commence *de novo* in England 25-30 years subsequent to return from the tropics.

* Occasionally in young adults. R. H. MILLER (1933) described a case in a boy of 11½ years of age; the writer in boys of 13 and 18. In this series, 67 per cent. of the cases were in males, 33 per cent. in females. The average age at onset works out at 45·3 years (maximum 72 years, minimum 18 years).

TABLE II.—*continued.*

DIFFERENTIAL DIAGNOSIS.

Idiopathic Steatorrhoea.	*Pellagra.* (Larval cases common).
Age. 20-60 years.	*Age.* 20-50, but may commence in infancy.
Distribution. Mostly Northern Hemisphere.	*Distribution.* Mostly tropics and subtropics in ill-nourished people.
Cause. Allied to, or sequel of " coeliac diseases " in infancy. Developmental defect of fat absorption. Achlorhydria rare.	*Cause.* Deficiency disease of dietetic origin. Deficiency of PP factor (vitamin B_2). Frequently secondary to chronic intestinal diseases, such as intestinal tuberculosis, bacillary dysentery and sprue. Achlorhydria in 40 per cent. of cases. Achylia rare.
Course. Onset insidious. Glossitis and dysphagia common. Wasting extreme. Diarrhoea frequent : steatorrhoea. 50 per cent. fat mainly split—soaps. Megacolon common. Changes in central nervous system uncertain. Tetany and cramps frequent. Pains in bones—kyphoscoliosis. Genu valgum : osteoporosis. Fractures. Clubbing of fingers, lens opacities. Infantilism in severe grades. Absorption affected by fat metabolism. Tetany common. Tendency to haemorrhages	*Course.* Onset insidious. Affected by sun's rays ; spring recrudescences. Glossitis and dysphagia common. Wasting moderate. Prodromal symptoms common. Diarrhoea frequent + dyspepsia. Stools, bilious, may be fatty—usually superadded intestinal infection. Eruption — dermatitis — hands, face, arms, scrotum—always symmetrical. Changes in central nervous system resemble subacute combined degeneration. Mental symptoms, irritability, excitability, melancholia. No osteoporosis, but fragility of bones.
Pathology. Megalocytic, hyperchromic anaemia and leucopenia, or simple hypochromic anaemia. Bilirubinaemia absent. Flat blood glucose curve. Hypocalcaemia extreme.	*Pathology.* Megalocytic, hyperchromic anaemia and moderate leucopenia in a proportion of cases. Bilirubinaemia absent. Blood glucose normal. Blood calcium normal. Urine = porphyrins in excess.
Treatment. Responds to some extent to liver in large amounts, vitamin B_2 and vitamin D.	*Treatment.* Responds to vitamin B_2—Nicotinic acid and riboflavin.
Relapses. Spontaneous remissions of short duration.	*Relapses.* Spontaneous remissions tend to occur in untreated cases.

THE TREATMENT OF SPRUE WITH NICOTINIC ACID.

" Similia similibus curantur."

It is difficult to assess the value of any particular line of treatment in such a chronic slowly-progressing disease as tropical sprue, in which so many of the signs and symptoms are of an evanescent character, which furthermore, in the absence of drugs, responds to dietetic treatment and in which the more urgent symptoms may even remit on leaving the endemic zone of the disease. At least one of these unassessable factors can be excluded from those cases which commence *ab initio* in former tropical residents several years—it may be as long as 25 years (see Case 4, Table III)—subsequent to their return to a temperate climate, and which constitute roughly one-third of those cited in this paper.

The writer has endeavoured to relate in sequence the steps which led to the application of vitamin B₂ treatment to sprue which appeared to be a logical sequence of experience gained in pellagra and in allied conditions characterized by glossitis. It seemed reasonable to suppose that this vitamin might not only exert an influence upon the outstanding clinical signs of sprue, but that the results obtained might also shed some light upon the aetiology of this mysterious disease. Therefore, commencing in October, 1938, a series of twenty-four well-marked and typical cases of sprue have been treated with therapeutic doses of nicotinic acid and seven with riboflavin in addition.

The results of this treatment, as far as they can be assessed at present, are summarized briefly in the accompanying table (Table III). As might have been predicted, the most striking effect of nicotinic acid has been upon sprue glossitis and the rapid return of the taste-sense to normal. The subsidence of these irritating symptoms undoubtedly exerts a profound influence upon digestion and assimilation which is most gratifying to the patient. It has been a common experience that on dietetic treatment reinforced by liver therapy, sprue glossitis with recurrent aphthous ulceration has formed one of the most annoying and intractable features of this disease, but the fiery redness of the tongue in advanced sprue tends to fade under nicotinic acid therapy within 24 hours, whilst the appearance is restored to normal within 3 or 4 days. In the glossitis of advanced sprue with marginal indentations and angular stomatitis (apparently identical with the pellagrous tongue), the addition of riboflavin would appear necessary to heal the angular excoriations, and it also has an effect on the epithelium of the lips. The cases have been followed up in so far as has been possible and in none of them has any recurrence of glossitis taken place as long as the nicotinic acid therapy has been sufficiently prolonged. The doses of nicotinic acid employed have varied in different cases from 150 to 300 mg. a day in tablet form and in seven cases this has been reinforced with 3 mg. of riboflavin daily. Probably further research may suggest that larger doses are advisable.

The effect of this treatment upon the intestinal manifestations has been less easy to gauge. It had been hoped to reinforce clinical observation by biochemical investigation upon the fat content of the stool, etc., but unfortunately, present circumstances have rendered this impossible. The impressions on the end result of treatment which have been gained are that the diarrhoea ceases within 4 days without the aids of any artificial means (colloidal kaolin, Batavia powder etc.) and that the stools become converted to normal size and colour in a period of 2 to 3 weeks from the commencement. This has been ascertained by weighing the amount of excreta passed daily and by comparing the results with those obtained by other methods of treatment during the last 20 years. It has certainly been possible to permit patients treated in this manner a greater range and variety of diet than was previously considered advisable and, moreover, the rapid return of the appetite and desire for food which is attributable to this treatment render this aim easier to attain.

If it is permissible to regard the condition of the tongue as a reflection of the state of the whole alimentary tract, then treatment which reacts upon the tongue should also exert a similar action upon the gastrointestinal mucosa and in some way influence absorption from the small intestine, to the lack of which the main signs and symptoms of sprue may be attributable. In this way then the effect of nicotinic acid upon digestion and assimilation may be best explained. Perhaps the most practical testimony of the efficacy of this treatment has been that no longer are strict and prolonged dietetic precautions necessary as heretofore. All the patients cited in this series have been able to partake of a normal diet within 6 weeks of being discharged from hospital and only one has had any return of sprue-like " reminders " since, and these ceased on resuming the treatment. This has been specially noticeable in the most advanced cases (see Table III—Cases 4, 5, 7, 8, 19, 20, 21 and 22). All have been able to resume active life and occupation. In their general appearance, mental attitude and capacity striking changes have occurred. One of the most advanced cases of sprue with anaemia returned to Ceylon (Case 19, Table III), over a year and a half ago, and is now quite well.

One of the most persistent and distressing aftermaths of sprue are flatulency and meteorism, but these features have been strikingly absent from my series. All those who have had experience of tropical sprue have remarked upon the disgruntled attitude of these patients, some of which is attributable to dietetic restrictions and others to the anaemia and irritating phenomena of this disease. A gratifying testimony to beneficial effects of treatment has been the more satisfactory mental outlook.

It is necessary to emphasize that nicotinic acid therapy must be prolonged for some considerable time—for three months, or even longer after the first preliminary bout. The writer has made a practice of advising the continuation of 150 mg. of nicotinic acid daily 14 days in each month for a six months

Case No.	Sex and Age	Country of Origin	Duration of Symptoms	Relapse or First Attack	Diarrhoea	Emaciation, loss in lb.	Glossitis and Angular Stomatitis	Anaemia	Test Meal
1	F. 37	India	2 years	First attack	+	14	+ +	R.B.C. 3,400,000 Hb. 80 per cent. megalocytic	Achlorhydria with response to histamine
2	M. 34	India	6 months	First attack	+ +	28	+ + +	R.B.C. 2,800,000 Hb. 70 per cent. megalocytic	Hypochlorhydria with response to histamine
3	M. 50	China	6 months	First attack	+	28	+ +	R.B.C. 4,800,000 Hb. 83 per cent. megalocytic	Hypochlorhydria with response to histamine
4	F. 56	Burma	1 year	First attack 25 years after leaving tropics	+ +	14 cramps & tetany	+ + + scrotal type of of tongue	R.B.C. 4,500,000 Hb. 82 per cent. megalocytic	Normal acid curve and response to histamine
5	M. 69	India	15 years	Fifth relapse	+ + + (15 years)	14	+ + (12 years)	R.B.C. 2,000,000 Hb. 78 per cent. megalocytic	Achlorhydria without response to histamine
6	M. 41	India	3 years	First attack	+ + 3 years	21	+ + 3 years	R.B.C. 5,000,000 Hb. 100 per cent. slight anisocytosis	Normal acidity with response to histamine
7	M. 66	India	4 years	Second attack	+ + 4 years	14 2 years	+ +	R.B.C. 4,600,000 Hb. 88 per cent. megalocytic	Hypochlorhydria with response to histamine
8	F. 05	China (Shanghai)	11 years	Fifth or sixth relapse	+ + 11 years	28	+ + + 10 years	R.B.C. 3,600,000 Hb. 80 per cent. megalocytic anaemia	—
9	M. 64	India	2 years	First attack 10 years after leaving India	+ + 2 years	28	+ + 4 months	R.B.C. 4,000,000 Hb. 90 per cent. megalocytic	Hypochlorhydria with response to histamine
10	M. 48	Malaya	5 months	First attack	+ +	21	+ + 4 months	R.B.C. 4,700,000 Hb. 90 per cent. anisocytosis	Hypochlorhydria with response to histamine
11	M. 35	Ceylon	5 years	First attack	+ +	14	+ + 6 months	R.B.C. 4,730,000 Hb. 96 per cent. anisocytosis	Hypochlorhydria with response to histamine
12	F. 45	China	3 years	First attack secondary to bacillary dysentery	+ + 3 years	14	+ + + 2 years	R.B.C. 4,110,000 Hb. 90 per cent. megalocytic	Hypochlorhydria with response to histamine
13	F. 60	India	13 years	Third relapse	+ + 13 years	20	+ + 6 years	R.B.C. 4,080,000 Hb. 74 per cent. megalocytic	Achlorhydria with response to histamine
14	M. 43	Malaya	3½ months	First attack	+ + 3½ months	14	+ + + 1 month	R.B.C. 4,920,000 Hb. 90 per cent. slight anisocytosis	Hypochlorhydria with response to histamine

III

GLOSSITIS AND DIARRHOEA TREATED WITH NICOTINIC ACID

Blood Calcium.	Faeces Fat.	Stay in Hospital.	Nicotinic Acid and Riboflavin.	Other Treatment.	Result.
10 mg. per 100 c.c.	21 per cent.	37 days	150 mg. daily for 17 days	i.m. injection, campolon + examen 20 c.c.	Glossitis subsided in three days, cessation of diarrhoea. Dark coloured faeces.
11 mg. per 100 c.c.	24 per cent.	22 days	150 mg. daily for 14 days	i.m. injection examen 44 c.c.	Tongue became less tender in 24 hours. Fifth day subsided with return of taste, cessation of diarrhoea.
10.3 mg. per 100 c.c.	30.7 per cent.	17 days	150 mg. daily for 10 days	i.m. injection campolon 16 c.c.	Rapid improvement in tongue in 48 hours. Improvement in size and colour of stools.
10.8 mg. per 100 c.c.	25 per cent.	40 days (surgical operation, also thyroid cyst)	150 mg. daily for 12 days	Surgical operation for thyroid cyst	Tongue normal in three days. Return of taste. Stools became brown and solid within ten days.
10 mg. per 100 c.c.	?	21 days (some degree of rectal prolapse)	150 mg. daily for 21 days	Liveroid ½ oz., b.d. by mouth	Rapid improvement of appearance. Return of tongue to normal. Cessation of diarrhoea and brown stools.
11 mg. per 100 c.c.	28 per cent.	12 days	300 mg. daily for 12 days	Rectal polypus removed	Tongue returned to normal in 48 hours. Cessation of diarrhoea. Brown stools in five days.
11.6 mg. per 100 c.c.	?	22 days	150 mg. daily for 12 days	i.m. injection campolon 10 c.c.	Tongue normal in 48 hours. Return of taste. Formed and brown stools in four days.
10 mg. per 100 c.c.	?	24 days	150 mg. daily for 10 days	i.m. injection campolon 24 c.c. Septic teeth extracted	Tongue became normal and diarrhoea ceased in four days.
11 mg. per 100 c.c.	26.5 per cent. stool weighed 52 oz.	33 days	150 mg. daily for 12 days	i.m. injection campolon 16 c.c.	Tongue became normal in four days. Stools brown and firm in six days.
11 mg. per 100 c.c.	30 per cent. stool weighed 40 oz.	15 days	150 mg. daily for 12 days	Septic teeth extracted	Tongue became normal in 48 hours. Stools improved within seven day.
10.5 mg. per 100 c.c.	Stool weighed 40 oz.	19 days	150 mg. daily for 12 days	i.m. injection campolon 64 c.c.	Tongue became normal in three days. Improvement in colour and size of stools.
10 mg. per 100 c.c.	22 per cent. fat	19 days	150 mg. daily for 14 days	i.m. injection campolon 40 c.c.	Glossitis subsided in two days. Faeces brown in four days.
?	?	28 days	150 mg. daily for 10 days	i.m. injection campolon 24 c.c.	Glossitis subsided. Stools improved in colour in six days.
10.5 mg. per 100 c.c.	38 per cent. fat	21 days	150 mg. daily for 14 days	No other treatment	Did well. Effect on tongue noted in four days. Diarrhoea ceased.

TABLE III

Case No.	Sex and Age	Country of Origin	Duration of Symptoms	Relapse or First Attack	Diarrhoea	Emaciation, loss in lb.	Glossitis and Angular Stomatitis	Anaemia	Test Meal
15	M. 53	India	4 months	First attack 4 years after arrival in England	+++ 4 months	14	++ 1 month	R.B.C. 2,000,000 Hb. 50 per cent. megalocytic + normoblasts +	Achlorhydria with response to histamine
16	M. 42	Burma	3 months	First attack 2 years after arrival in England	++ 3 months	20	++ angular stomatitis 2 months	R.B.C. 3,800,000 Hb. 70 per cent. megalocytic	Achlorhydria with response to histamine
17	M. 71	Burma	10 years	Third relapse 10 years after leaving Burma	++ 10 years	42	+++ Beefsteak tongue + angular stomatitis resembled pellagra stomatitis	R.B.C. 4,650,000 Hb. 95 per cent. megalocytic	Achlorhydria with response to histamine
18	F. 48	China (Shanghai)	10 years	Fourth relapse 10 years after leaving China	++ 10 years	14	Tongue red, raw and angular stomatitis	R.B.C. 3,000,000 Hb. 80 per cent. megalocytic	?
19	M. 52	Ceylon	2 years	First attack	++ 1 year	28	Tongue red, raw with angular stomatitis	R.B.C. 1,800,000 Hb. 40 per cent. leucopenia 3,300 megalocytosis, anisocytosis, basophilic stippling, Price-Jones curve flattened type	Achylia gastrica—no response to histamine X-ray of stomach showed gastritis
20	M. 42	Burma	3 months	First attack	+ vomiting	14	++	R.B.C. 3,000,000 Hb. 70 per cent. megalocytic	Achlorhydria
21	M. 71	Burma	10 years	Many relapses	++	14 low blood pressure	++ and angular stomatitis	R.B.C. 3,500,000 Hb. 80 per cent. megalocytic	?
22	M. 55	India	5 months	First attack	++	42	+ and loss of taste and smell	R.B.C. 2,470,000 Hb. 60 per cent. megalocytic	Achylia gastrica
23	M. 42	Burma	3 months	First attack	+	20	++ and aphthae	R.B.C. 3,500,000 Hb. 80 per cent. megalocytic; coincident B.T. malaria	Achlorhydria

—(continued).

Blood Calcium.	Faeces Fat.	Stay in Hospital.	Nicotinic Acid and Riboflavin.	Other Treatment.	Result.
9·5 mg. per 100 c.c.	52 per cent. fat	19 days	150 mg. daily for 12 days	i.m. injection campolon 20 c.c. 4 teeth extracted	Did well. Adequate reticulocyte response to 10 per cent. Glossitis abated in four days.
?	? weighed 8 to 16 oz.	21 days	300 mg. daily for 14 days, riboflavin 9 mg. for 7 days	i.m. injection campolon 30 c.c.	Immediate improvement in tongue and return of taste sense. Riboflavin healed angular stomatitis and also excoriation round anus.
9·6 mg. per 100 c.c.	? weighed 3 to 36 oz.	14 days (increase in weight 7 lb.)	150 mg. daily for 14 days, riboflavin 9 mg. for 7 days	i.m. injection campolon 36 c.c.	Raw-beef tongue assumed normal colour in 48 hours. Sense of taste returned in four days. Appetite improved. Stools became smaller (8 oz.) and formed within seven days.
?	Weighed 7 to 9 oz., 31 per cent. fat	23 days (increase in weight 1 lb.)	150 mg. daily for 14 days	i.m. injection examen 24 c.c.	The tongue assumed a normal appearance in four days. The sense and taste of smell returned. The diarrhoea ceased and the stools became brown in colour within six days.
10 mg. per 100 c.c. + oral glucose curve flattened type	Weighed 12 oz. 30 per cent. fat, 51 per cent. split, 49 per cent. unsplit	37 days (increase of weight 3 lb.)	150 mg. daily for 21 days	i.m. injection of campolon, total 60 c.c.	The tongue assumed a normal aspect in five days. The pain disappeared and the taste returned. The stools became firm, of normal size and colour within ten days. A high reticulocytosis to 24 per cent. Colour became red and appearance totally changed. Three months afterwards R.B.C. 5,000,000, Hb. 100 per cent. Blood cells normal. Achylia gastrica persisted. Returned to Ceylon.
—	Fatty faeces, 16 oz.	19 days	150 mg. daily and 3 mg. riboflavin	i.m. injection of 5 c.c. campolon for 7 days	Glossitis and angular stomatitis subsided in three days, diarrhoea in twelve days. Improvement in colour and consistency of stools.
9·6 mg. per 100 c.c.	Fatty faeces, 16 oz.	14 days	300 mg. daily for 14 days, and 3 mg. riboflavin	i.m. injection of campolon 5 c.c. for 10 days	Glossitis and stomatitis subsided within three days, return of taste sense. Diarrhoea and meteorism ceased. Increase in weight 14 lb.
9·8· mg. per 100 c.c.	Large, fatty; 43 per cent. fat	70 days	300 mg. daily daily and 3 mg. riboflavin for 28 days	Blood transfusion 450 c.c.; i.m. injection 15 c.c. weekly Bencard liver extract	Glossitis healed in three days. Thereafter return of taste and smell. Diarrhoea ceased, stools returned to normal. Increase in weight 42 lb. Seen six months after—on full diet, no return of symptoms. The main interest in this case was the prompt response to vitamin B_2 treatment after previous failure of liver therapy.
?	Fatty stools 16 oz.	11 days	150 mg. daily and 2 mg. riboflavin	i.m. injection of campolon 2 c.c. for 7 days	4 lb. increase in weight in ten days. Aphthous ulcers healed in three days. Diarrhoea ceased. Stools became brown and normal within seven days. No return of sprue symptoms.

Intramuscular

period to prevent recurrences. It is not claimed that vitamin B_2 treatment exerts any influence upon haemopoiesis and consequently it is necessary, as in this series, to continue intensive parenteral liver therapy in conjunction with the specific treatment. No evidence has been obtained that in the absence of liver therapy a definite reticulocytosis can be obtained.

As regards toxic effects of nicotinic acid therapy, it can be said that they are remarkably few. The immediate results of chewing a 50 mg. tablet of nicotinic acid is to produce a histamine-like effect with flushing of the face and neck and a sense of warmth. In some a tingling of the jaw along the course of the inferior dental nerve has been observed, and in one a roseolar rash over the trunk and abdomen which persisted for three days was attributed to it.

Finally, it should be emphasized that this form of therapy has an effect upon the skin of advanced cases of sprue. The harsh, dry, scaly and inelastic skin resembling old parchment usually forms a salient feature. One of the most noticeable effects which has become obvious to the patients themselves has been the rapid improvement in the appearance and texture of the skin coincident with increase in body weight which has been universal in this series.

It is too early as yet to assess the value of this line of treatment in preventing future relapses which in the past have formed such a feature of tropical sprue. The writer may claim to have had special experience in the treatment of this disease, and in his opinion, no other method has given such striking results as have been obtained by nicotinic acid therapy.

It is therefore eminently desirable that the indications here set forth as the result of clinical observations should be offset by careful estimations of the vitamin absorption and excretion which can be performed solely as the result of elaborate laboratory investigations.

To the summaries in Table III may be added a twenty-fourth case in which the response to nicotinic acid therapy has been so striking as to permit a few more details.

A woman of 59 was found to have advanced sprue which had long remained unrecognized. For 15 years she had been suffering from sprue symptoms and for 5 had been subjected to prolonged distress and pain from an intense red, raw and bleeding glossitis with aphthous ulceration and angular stomatitis. Latterly malignant disease had been suspected and the outlook was regarded as hopeless. There was advanced megalocytic anaemia with scanty megaloblasts. The abdomen was grossly distended and her suffering from flatulence was great ; cramp in the legs and tetany had been noted from time to time. The skin was inelastic, dry and scaly. The stools were pale, greasy and copious. Wasting was extreme and she had lost 56 lb. in weight over a period of 5 years. There existed some doubt as to where exactly the disease had been contracted. Twenty years ago she had lived for 2 years in Singapore and till 1938 had been in Beira, Portuguese East Africa.

The response to nicotinic acid therapy was prompt and gratifying (nicotinic acid 300 mg. plus 3 mg. riboflavin daily for 4 weeks). Within 4 days the rawness and sensitiveness of the tongue had disappeared, and within 16 days its appearance was normal and with it the filiform and fungiform papillae were regenerated. The cessation of the diarrhoea was almost immediate ; within 4 days the stools became formed and brown and within

5 weeks they were normal in size, consistency and colour. The appetite is now good; weight has increased by 18 lb. and the blood picture has improved out of all recognition. She is now on the high road to complete recovery.

NON-TROPICAL SPRUE.

The possibility of the occurrence of sprue, or a sprue-like disease, usually of a subacute type, resembling in clinical appearances and course true tropical sprue, but distinct from idiopathic steatorrhoea, has been foreshadowed by many students of this subject in Denmark, Holland and the United States. THAYSEN (1932) in his monograph described in considerable detail " non-tropical sprue," which he considered to be identical with the classical disease emanating from tropical countries. The writer in 1929 described as " non-tropical " or " indigenous " sprue any case originating in England which from its general behaviour and response to dietetic treatment corresponded to the tropical disease ; but at that time the clinical status of coeliac disease and idiopathic steatorrhoea had not been accurately defined. Recently, however, the question has again been raised by BENNETT and HARDWICK (1940) who have encountered two such cases in England responding like tropical sprue to nicotinic acid therapy. Since that time the writer has diagnosed and treated two cases which appear to fall into this category. Both had no ascertainable connection with the tropics and had either been examined solely from the tropical viewpoint the diagnosis of tropical sprue would probably have been arrived at. The response to nicotinic acid was in each instance most satisfactory.

Cases of Non-Tropical Sprue.

(1) A man of 56, a Pole by birth, who had resided in Lambeth, London, for 42 years and had been suffering from fatty diarrhoea, glossitis and emaciation for 2 years. Various diagnoses had been made and recently abdominal neoplasm had been suspected. There was generalized glossitis with angular stomatitis, extreme meteorism with a sprue-like abdomen and slight megalocytic anaemia. The stools had been large, offensive, pultaceous, and fatty ; diarrhoea had been continuous—four to five motions a day. On nicotinic acid (300 mg. daily) and riboflavin (3 mg. daily) therapy the diarrhoea ceased within 4 days and the glossitis cleared up. There was a gratifying increase of weight, and when seen a month afterwards the appearance of the patient had entirely changed.

(2) This patient, a male, was 64 years of age and presented the cachectic appearance of advanced sprue. He had suffered from glossitis and stomatitis with fatty diarrhoea for 8 years and so great was the emaciation that a gastric carcinoma had been suspected. There could be little doubt from the appearance of the patient, acute glossitis with angular stomatitis and macrocytic anaemia, abdominal distension and pale fatty faeces that the correct diagnosis was sprue, but the only possible connection with the tropics was a short stay of 7 days in Egypt 15 years previously, 5 years before the onset of symptoms. It did not therefore appear possible to connect his illness with his residence in that country.

The progress of this case has been consistently good. Whilst maintaining an active life and living on a varied and generous dietary, his weight increased rapidly, and the last reports I have obtained are to the effect that he has resumed a busy and exacting occupation. (See Weight Chart, p. 368.)

It is necessary to add that there was no bony lesion in either case or any other feature suggestive of idiopathic steatorrhoea.

WEIGHT CHART.
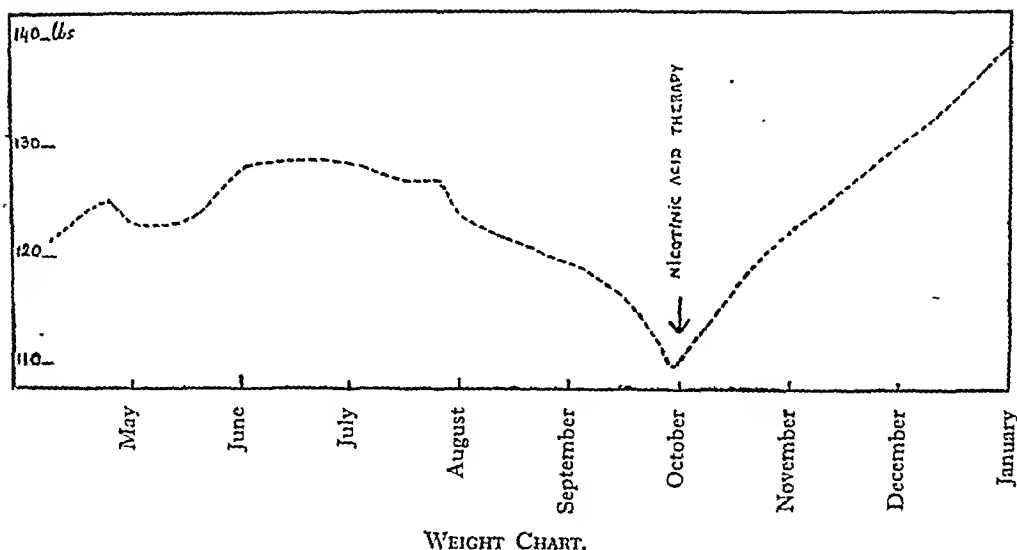
SUMMARY AND DISCUSSION.

The grounds upon which the vitamin B₂ treatment of sprue are based may be summarized as follows :—

1. The comparison which may be drawn between the principal manifestations of sprue and pellagra, more especially as regards glossitis and stomatitis.

2. The almost instantaneous reaction of the sprue glossitis (as in pellagra) to nicotinic acid therapy.

3. The general response of well-marked sprue to vitamin B₂ as shown by its effect upon the glossitis and the return of the intestinal functions to normal, which raises the point as to whether the changes in the tongue are not of essentially the same nature as those in the bowel.

The hypothesis is advanced that the sprue syndrome is mainly due to non-absorption, or destruction of, vitamin B₂ in the small intestine.

The writer has already emphasized that macroscopic lesions of the ileum may produce the clinical appearance of sprue, whilst more recently BENNETT and HARDWICK (1940) have ably discussed a clinical state designated as " chronic jejuno-ileal insufficiency," showing that in the failure of the small intestine to perform its normal functions, glossitis, steatorrhœa, emaciation, meteorism, megalocytic anaemia, hypocalcaemia and tetany result : all of which are typical of sprue. They have shown moreover that some of these, such as the glossitis of gastro-jejuno-colic fistula,* react to nicotinic acid therapy.

It may therefore be assumed that the disease which we recognize in tropical medicine as sprue represents the fully developed picture of small intestine

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*The same obtains following upon the unsatisfactory results of gastro-enterostomy in which there is intestinal hurry.

deficiency and is presumably due to previous damage to the intestinal mucosa. That the main lesion of sprue is confined to the small intestine is suggested by the diaphanous appearance postmortem of this viscus in advanced cases, as well as by the abundantly proven clinical observation that abdominal distension is due to inflated coils of the intestine. If the signs and symptoms of tropical sprue can be attributed to an avitaminosis B_2, then the nature of the basic process can only be surmised at present. It now becomes extremely difficult to understand why this failure to absorb vitamin B_2 should be confined mainly to European residents of certain parts of the tropics, sometimes becoming manifest many years after quitting the supposedly endemic areas. Why indeed should an avitaminosis B_2 be solely confined to the tropics ? In the writer's opinion the problem of the aetiology of sprue has assumed a fresh complexion, and it may even be questioned whether " sprue " is indeed solely a tropical disease. On the analogy with pellagra in which the typically developed disease is mainly tropical or subtropical in distribution on account of the dietetic and other conditions obtaining there, it is possible to postulate that, though the sprue syndrome is commonly met with in its most typical and fully developed form in tropical residents, yet minor manifestations of the same process may be encountered elsewhere as in northern European countries and in the United States and is known as "non-tropical sprue". The writer is convinced that cases of this nature are distinct from coeliac disease and idiopathic steatorrhoea in that they react like tropical sprue to nicotinic acid therapy. In the acceptance of this new idea it is of course necessary to recognize that emaciation, the degree of anaemia, tetany, diarrhoea and steatorrhoea probably depend upon the extent to which the absorptive surface of small intestine is involved. This conception may account for some of the cases described by THAYSEN as " non-tropical sprue " and the two cases published by BENNETT and HARDWICK (1940). Recently the writer has studied two similar cases (cited above) exhibiting the sprue syndrome : both had had no obvious association with tropical conditions and both showed a gratifying and satisfactory response to nicotinic acid therapy.

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LYSIS OF BLOOD OF MALARIA PATIENTS BY BILE OR BILE SALTS

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The etiology of blackwater fever has been a subject of discussion for many decades. There is general agreement today that malaria infection is the primary cause; but the immediate cause which produces the crisis—the explosive destruction of blood cells, is still a matter of surmise. KLIGLER (1923) approached the problem on the assumption that the cells of malaria patients are more sensitive to lysis and hence are more readily destroyed by a combination of excess bile in the circulation, due to liver injury, and quinine. He called attention to the fact that in chronic malaria patients jaundice is always a premonitory sign of haemoglobinuria, while experimentally he demonstrated (1) that dilutions of bile and quinine which separately had no effect on blood cells caused prompt laking when used in combination and (2) that blood of malaria patients was more sensitive to lysis than normal blood. Rabbit blood reacted to quinine and bile in the same way as normal human blood.

More recently PONDER and ABELS (1936) showed that addition of quinine to lytic systems containing saponin or sodium taurocholate produces marked acceleration of haemolysis of rabbit blood cells, thus confirming our own results. These authors showed further that administration of quinine to rabbits rendered the cells more sensitive to lysis, the rate depending on the amount of quinine injected. Small doses (20 mg./kg.) for 15 days had no effect; larger doses (50 mg./kg.) reduce resistance two to four-fold.

No systematic study has yet been made of the sensitivity of blood cells of malaria patients to lysis. VINCENT (1906) was the first to observe that a lower concentration of quinine sufficed to lake blood of malaria patients than that of normal people, and in the work cited above KLIGLER (1923) showed that malaria blood was more readily laked by the bile-quinine system than control normal

blood. The observations reported below were carried out for the purpose of ascertaining more carefully the relative sensitivity of normal and malaria blood to a given lytic system.

PROCEDURE.

The technique was simple: all quantities were constant, the only variable being the time required for haemolysis. Into tubes 6 mm. in diameter were placed 0·3 c.c. of a 0·3 per cent. saline solution of dried ox-bile (Difco) and one drop of fresh blood (about 0·08 c.c.). The blood was uniformly suspended by shaking and the mixture incubated at 37° C. until haemolysis was complete. The relative sensitivity or resistance of the cells was measured by the time required for complete haemolysis. A suspension of cells in saline served as control.

RESULTS.

Blood from healthy individuals who had never had malaria, and blood from malaria patients before and after treatment was tested in the manner described above. Blood from normal individuals, either different ones, or the same people tested at different times, gave haemolysis in 4½ to 5 hours. Of sixteen blood samples tested ten were laked in 5 hours and six in 4½ hours. Blood of two normal individuals tested three and four times respectively at intervals, always laked after 5 hours. This period may, therefore, be accepted as the normal laking time in the system used.

We next tested the laking time of blood taken from malaria patients before treatment. The data are given in Table I. It will be noted .that early in the attack (during the first four days), the resistance of the cells may or may not be

TABLE I.

LAKING TIME (IN HOURS) OF RED CELLS FROM UNTREATED MALARIA PATIENTS, AT VARIOUS INTERVALS AFTER ONSET OF THE ILLNESS.

Cases.	Days of Illness.	Time of Haemolysis.
1	2	3½
2	3	5
3	4	5
4	4	3½
5	7	2¾
6	21	2½
7	21	2½
8	21	2¾
9	25	2
10	30	2

lowered and, if decreased, then only to a moderate degree. Blood taken seven days after the onset or during relapses showed a markedly reduced resistance of

TABLE II.

HAEMOLYSIS RATE OF RED BLOOD CELLS FROM MALARIA PATIENTS UNDERGOING TREATMENT.

Days Ill.	Days Treated.	Treatment.	Time of Complete Haemolysis (hours)
7	5	quinine	$\frac{1}{2}$
6	5	,,	$\frac{1}{2}$
3	2	,,	1
4	2	,,	1
2	7	,,	$1\frac{1}{2}$
5	3	,,	$1\frac{1}{2}$
10	8	,,	$1\frac{1}{2}$
?	3	,,	$1\frac{1}{2}$
25	4	,,	$1\frac{1}{2}$
29	4	quinine + plasmoquine	$1\frac{1}{2}$
32	7	,, ,,	$1\frac{1}{2}$
?	6	quinine	2
?	2	,,	2
6	3	,,	2
?	7	,,	2
5	?	,,	2
64	4	,,	2
8	4	,,	2
5	3	,,	2
8	6	,,	2
7	5	,,	$2\frac{1}{2}$
2	2	,,	$2\frac{1}{2}$
6	2	,,	$2\frac{1}{2}$
?	1	,,	$2\frac{1}{2}$
?	4	,,	$2\frac{1}{2}$
11	8	,,	$2\frac{1}{2}$
14	9	,,	$2\frac{1}{2}$
10	4	,,	$2\frac{1}{2}$
14	8	quinine + plasmoquine	$2\frac{1}{2}$
17	11	,, ,,	$2\frac{1}{2}$
9	4	quinine	$2\frac{1}{2}$
30	9	quinine + plasmoquine	$2\frac{1}{2}$
36	15	,, ,,	$2\frac{1}{2}$
?	10	quinine	3
8	5	,,	3
?	3	,,	3

the red cells, the laking time being decreased from 5 to $2\frac{1}{2}$ or 2 hours. In none of these cases was there a reduction in the red cell count.

The next series of tests was made on blood from malaria patients undergoing treatment. These results are given in Table II. In this table are given the total duration of the illness, duration of the present course of treatment, and the drug used. If these results are compared with those in Table I it becomes evident that treatment with quinine alone as well as with quinine plus plasmoquine cause a further more or less marked decrease in the resistance of the red cells to laking. The relative sensitivity varies with individuals rather than with the duration of the treatment. This fact may be of significance in relation to the so-called host factor or host predisposition to haemoglobinuria. It should be noted that whereas the average laking time for malaria blood of untreated patients was 3·1 hours, that of blood taken during treatment was 2·0 hours. The range in the first group was 2 to 5 hours, that in the second group was $\frac{1}{4}$ to 3 hours.

In a fourth group of individuals the laking time was determined in the same individuals either before and during treatment, or at intervals during treatment. The data are shown in Table III. It will be noted that in nearly every case tested the treatment was associated with a decreased resistance of the red cells to laking, irrespective of the type of parasite responsible for the infection.

A final group of ten normal individuals was given 0·6 gramme quinine *per. os* for three days and the blood tested before and after the administration of quinine. No appreciable change in the resistance of the cells was noted. In all but one case the laking time was the same before and after and in all cases it was $4\frac{1}{2}$ to 5 hours—the same time as that recorded above for normal blood.

Discussion of Results.

The etiology of haemoglobinuria still requires elucidation. The various sporadic studies hitherto reported have been suggestive and have indicated two important points, (1) that blood of malaria patients is more sensitive to lysis than is normal blood (VINCENT, 1906, KLIGLER, 1923); and (2) that quinine and bile further accelerate the rate of haemolysis (KLIGLER, 1923, PONDER and ABELS, 1936). It seemed apparent that this path of investigation deserved more thorough exploration than has been accorded it hitherto. In this preliminary report we have not only been able to confirm the previous studies but have also obtained results which suggest that there are considerable individual variations in loss and return of resistance during treatment. Moreover, it is obviously necessary to ascertain whether the lowered resistance of red cells is the same or different in different types of infections. It will also be of interest to establish whether sub-icteric conditions in themselves or in association with quinine render the cells of malaria patients or of those with other ailments more sensitive to lysis. It appears to us that studies such as those indicated may throw light on the mechanism of malaria haemoglobinuria.

TABLE III.

LAKING TIME OF RED CELLS OF MALARIA PATIENTS BEFORE AND DURING TREATMENT.

Diagnostic Findings.	Days before treat-ment.	Time of Complete Haemolysis in relation to days of treatment														
		Day of Treatment														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. M.A. Age 20; 25 days' ill; quartan sporulating bodies, gametocytes, rings; temperature, 38·5° C.; R.B.C., 4·8; haemoglobin, 90 per cent.; spleen, 1; liver—; urine urine protein —, bilirubin —	2				1½ hrs			1½ hrs								
2. S.M. Age 35; 4 days ill; M.T. rings + + +; temperature, 36·2° C.; R.B.C. 5·4; haemoglobin 90 per cent.; spleen 2½ fingers; liver 2 fingers; urine: protein +, bilirubin + + +. After 4 days' treatment: protein —, bilirubin —	5				2 hrs											
3. K.A. Age 35; 21 days ill; M.T. gametocytes +; temperature 36·6° C.; R.B.C. 4·95, haemoglobin 85 per cent.; spleen —, liver 1-2 fingers; two examinations before treatment (on two consecutive days). After 15 days treatment, R.B.C. 5·1	2½,	2½ hrs			1½ hrs					2½ hrs						2½ hrs
4. A.M. Age 20; 3 days ill; B.T. rings; schizonts + +; temperature 36° C.; R.B.C. 5·1; haemoglobin 90 per cent.; spleen —, liver —	5					3 hrs										
5. R.S. Age 25; 2 days' ill; M.T. rings + +; temperature 39·7° C.; R.B.C. 5·0; spleen —, liver —	3½				1½ hrs				1½ hrs							
6. Sch. Age 18; 5 days' ill; M.T. rings + +; temperature 36° C.; R.B.C. 4·9 per cent., haemoglobin 80 per cent.; spleen —, liver —					2 hrs				2 hrs							
7. Sch. D. Age 40; 7 days' ill; M.T. rings + + +; temperature 36·9° C.; R.B.C. 4·0, haemoglobin 75 per cent. After 4 days' treatment, M.T. rings, gametocytes; after 7 days' treatment, M.T. gametocytes + +; after 11 days treatment, M.T. gametocytes					2½ hrs		2½ hrs					2½ hrs				

of the well known leakage of chloride in the evacuations.   This is due to a great concentration of the blood in cholera cases.   About the changes found in the very severe cases ROGERS may be quoted : " For the most severe fatal cases in which on the average two-thirds of the fluid of the blood has been lost, the chlorides in the blood were slightly lower than normal instead of being three times as great as would have been the case if no salts had disappeared from circulation."

It is, therefore, very necessary to remember the fact of this blood-concentration in any biochemical study of the blood in cholera.

The present investigation consists of a study of the different constituents of the blood in the cholera patients of the Carmichael Medical College, Calcutta, during the summer epidemic of 1939 in 105 cases of clinical cholera, many of the observations being repeated from day to day.

The Bases.—The sodium of the blood serum showed greatly reduced figures especially when the blood concentration is taken into account.   After the administration of saline transfusions, as would be expected, there was a normal or even a high sodium content.

These statements will be apparent from the following table.

TABLE I.

SHOWING THE RELATION OF THE SODIUM CONTENTS OF THE PLASMA.

Case.	Date.	Specific Gravity of Blood.	General Remarks.	Sodium mg. per 100 c.c. Serum.
103	3–6–39	1060 R.B.C. 6·8 millions	Before administration of saline	299
	6–6–39	1058 R.B.C. 5·2 millions	After administration of 3 pints of saline Hypertonic 1 pint (intravenous) Hypotonic 1 ,, ,, Normal saline 1 pint (subcutaneous)	336
104	3–6–39	1060	Before administration of saline	286
	8–6–39	1056	After administration of 4 pints of saline Hypertonic 1 pint (intravenous) Hypotonic 1 ,, ,, Normal saline 2 pints (subcutaneous)	325

The normal figures may be given here for comparison, that of PETERS, VAN SLYKE, HALD and EISENMAN (PETERS and VAN SLYKE, 1932), being 308 (nineteen cases) and of ours (thirty cases) being 328 mg. per 100 c.c. serum.

Potassium.—The normal figures for the potassium content of the blood serum are given.

That of PETERS, VAN SLYKE, HALD and EISENMAN (*loc. cit.*), is 18·3 mg. (nineteen cases) and that of ours (CHATTERJEE and SEN GUPTA, 1939) is 20·4 (fifteen cases).

The cholera cases show very little or no depletion of potassium but rather a high figure of this constituent in severe cases. The average of our 105 cases being 25·15. This figure is just a mathematical average, no consideration being given to the concentration of the blood or administration of saline. The blood findings of a few cases are given below.

It would be seen that in some cases the potassium was high and tended to be normal after administration of saline. This may be regarded as an after-effect of dilution. But in others even after administration of large amounts of saline and consequent lowering of specific gravity of the blood, the blood potassium tended to be high (Table II). It may be pointed out that none of the saline solutions contained any potassium salts.

TABLE II

SHOWING THE RELATION OF THE POTASSIUM CONTENT.

Case.	Date.	Specific Gravity of Blood.	General Remarks.	Potassium mg. per 100 c.c. Serum.
91	29–5–39	1056	After administration of 4 pints of saline Hypertonic 2 pints (intravenous) Hypotonic 1 „ „ Normal saline 1 pint (subcutaneous)	25·4
	31–5–39	1054	Convalescent	19·2
79	15–5–39	1056	After administration of 3 pints of saline Hypertonic 1 pint (intravenous) Hypotonic 1 „ „ Normal saline 1 pint (subcutaneous)	35·0

Calcium.—The average figure for calcium in normal Indians as found by us is 9·5 mg. per 100 c.c. serum (thirty cases). The study of the blood calcium shows that there is a definite lowering of the serum calcium. This appears to be very pronounced when the specific gravity of the blood is taken into account.

It also appears that with clinical improvement of the patient as the specific

gravity of the blood becomes normal the serum calcium improves greatly in amount.

It is difficult to localize the source from which the increased calcium is mobilized especially in view of the fact that no calcium is given to the patients either with the saline transfusions or per mouth except perhaps as negligible impurities. A few illustrative examples are given below in Table III.

TABLE III.

BLOOD CALCIUM IN CHOLERA CASES.

Case.	Date.	Specific Gravity of Blood.	Clinical Conditions.	Blood Calcium in mg. per 100 c.c. Serum.
82	18–5–39	1069	Before administration of saline	8·4
	20–5–39	1060	After transfusion of 4 pints of saline	12·0
90	25–5–39	1068	Before administration of saline	10·0
	27–5–39	?1054	After 11½ pints of saline transfusion	13·3
	31–5–39	1054	No further saline given	12·0
103	3–6–39	1060	Before administration of saline	10·6
	6–6–39	1058	After 4 pints of saline transfusion	11·6

Magnesium.—We have not been able to find any constant changes in the cholera patients' blood serum ; most of the readings being within the normal range, the figures sometimes being higher, at other times lower, than the normal average.

The normal figure given by PETERS, VAN SLYKE, HALD and EISENMAN (nineteen normal cases) is 2 mg. per 100 c.c. serum. The figure obtained by us is 3·3 mg. per 100 c.c. serum in thirty normal cases.

Chlorides.—As early as 1850 SCHMIDT showed that the serum of cholera patients contained less than the usual concentrations of chloride and sodium. EDMUND PARKES (1849) showed that the rice-water stools of cholera contain ½ to 1 per cent. of salts and very little albumin. ROGERS (1921) found an average of 0·53 per cent. of chlorides in the rice-water stools of cholera patients although there was very small amount in the watery material vomited from the stomach. In contrast to this, the chloride loss in the normal stool is insignificant as has been observed by HOLT, COURTNEY and FALES (1913). The therapeutic replacement

of the fluid and chlorides by saline transfusions may by this time be termed classical and need not be recounted here. The question as to which of the two portions, *viz.*, the sodium base or the chloride, is lost in greater amounts is of more than theoretical interest. It would appear that in some of the cases at least the loss of sodium base is more marked than the loss of the chloride. This will be apparent from Table IV.

TABLE IV.

RELATION OF SODIUM AND CHLORIDE IN THE SERUM AND BLOOD.

Case.	Date.	Specific Gravity of Blood.	Sodium mg. per 100 c.c. Serum.	Chloride mg. per 100 c.c. Blood.
90A	25–5–39	1068	294	600
94	30–5–39	1058	275	433
104	3–6–39	1060	286	533

Blood Sugar.—We find in 75 per cent. of our cases the blood examined before the administration of saline showed a marked fall in the level of the blood sugar. A few cases are given below in Table V.

TABLE V.

SHOWING THE USUAL BLOOD SUGAR.

Case	Specific Gravity of Blood.	Blood Sugar per 100 c.c. Blood.
96	1066	0·07
92	1058	0·07
78	1058	0·05
82	1068	0·05

That there is a fall in blood sugar in shock-like conditions is a well known fact (PORGES, 1910; and SIMPSON, 1937). Besides, the great muscular cramps and intestinal movements may have a part in the production of this hypoglycaemia. In 25 per cent. of our cases the blood sugar level did not show any hypoglycaemia. Of these, four cases again showed what may be called figures above the normal average. Diabetes was excluded in this group as subsequent blood examinations

did not show the high blood sugar levels. The blood findings of such a case are given in Table VI.

It is quite possible that these comparatively high figures for the blood sugar may be a result of the concentration of blood and possible non-utilization of sugar due to some other causes.

The Nitrogenous Constituents.—SHORTEN (1918) observed an increase of urea nitrogen and non-protein in the blood of cholera patients.

TABLE VI.

BLOOD CHANGES IN A PATIENT WITH A COMPARATIVELY HIGH
BLOOD SUGAR LEVEL.

Case.	Date.	Blood Sugar.	Specific Gravity of Blood.
95	25–5–39	0·166 per cent.	1060
95	29–5–39	0·121 per cent.	1056

This has been further confirmed by ROGERS (1921), DHAR, DHAR and ADHYEE (1930) and BANNERJEE (1936).

That this nitrogenous increase and retention is not due to concentration of the blood will be observed by the fact that even in those cases in which the specific gravity is restored there still remains the high nitrogenous retention, the figures being considerably high although reduced (Table VII).

The question of nitrogenous retention has been taken up in detail in a different study on cholera kidney.

TABLE VII.

Case.	Date.	Clinical Features.	Specific Gravity of Blood.	Urea.	N.P.N.
90H	25–5–39	Before saline	1068	35·0	44·8
	27–5–39	After usual saline treatment	1056	28·0	44·8
	31–5–39	After usual saline treatment	1056	21·0	36·4

Some Other Features.

Amongst the most important concomitant features of cholera is acidosis. Thus the classical observations made by ROGERS show a remarkable reduction of the alkalinity of the blood in cholera. He also demonstrated a close relation between the specific gravity of the blood and its diminished alkalinity in cholera.

SHORTEN's observations (*loc. cit.*) are similar. He has also demonstrated an increase of inorganic phosphates in the blood of cholera patients.

TSURUMI and TOYODA (1922) similarly observed a marked fall in alkali reserve in severe cases of cholera.

LIU, WANG and FAN (1933) found a constant acidosis in the acute stage. According to their findings there was a decrease in pH and in bicarbonate, a diminished carbon-dioxide content, a distinct reduction in serum total base and chloride concentration. There was an elevation of phosphate lactate and, to a lesser extent, the proteins.

But although this acidosis of cholera is a very common feature—the same is found in various other conditions of shock.

CANNON (1918) found a marked decrease in alkali reserve in cases of shock due to battle wounds, of severe haemorrhage and of gas bacillus infection. McELLROY (1918) found a decrease in reserve alkalinity in experimental shock. He has shown that the maintenance of alkali reserve at normal levels by injections of sodium bicarbonate solution did not prevent the development of shock. He concluded that acidosis is not a cause of shock but rather a secondary associated condition. The Special committee for the study of shock and allied conditions under the Medical Research Council investigated the relation between acidosis and shock. Their conclusion drawn from experimental and clinical evidence may be quoted " A progressive fall of the alkali reserve is the result of an inadequate supply of the blood pressure to the tissues and is a symptom of deficient capillary circulation, not a cause of such."

CANNON (1918) ; CANNON *et al.* (1918) ; and McELLROY (1918) determined the carbon-dioxide combining power of the blood of wounded persons in shock. They found that there was no reduction of alkali reserve until the blood pressure fell to 90 or 80, below which the alkali reserve decreased proportionately to the fall in blood pressure.

The changes in the serum electrolytes, *viz.*, that observed with sodium, potassium and the chlorides has also been observed in cases of shock due to various causes (LOEB *et al.*, 1933).

Similarly the hypoglycaemic condition has also been observed in shock (PORGES, 1910 ; BRITTON, quoted by SIMPSON, 1937).

Methods.

For sodium we have adopted the method of DOROTHY ROURKE (1928) ; for potassium that of KRAMER and TISDALL (1921a) ; for magnesium that of HAURY's (1938) modification of the method of HIRSCHFELDER and SERLES (1934) ; for calcium that of KRAMER and TISDALL (1921b) ; for urea that of MUKHERJEE (1929) ; for chlorides that of VAN SLYKE (1923) ; for non-protein nitrogen, the micro-Kjeldahl method, and for blood sugar that of the modified method of FOLIN and WU (1920).

SUMMARY.

A. The blood of cholera patients shows :—

    1. A diminished sodium content of blood.

    2. An increased potassium content.

    3. Serum calcium is low but increases in clinical improvement even without its therapeutic replacement.

    4. A diminished chloride of the blood serum but the chloride leakage is proportionately less than that of sodium.

    5. A decrease in blood sugar, a few cases however do not show hypoglycaemia.

    6. An increase of urea and non-protein nitrogen.

B. The pathogenesis of diminished alkali reserve and acidosis is discussed.

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A NEW FORM OF ACUTE HAEMOLYTIC ANAEMIA.
"BAGHDAD SPRING ANAEMIA"

BY

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During the spring of 1939 my attention was drawn by several physicians to the fact that here in Baghdad a special form of acute anaemia occurs every year almost at the same time. The disease—so I was told—starts quite acutely, runs a very quick course, has a considerable mortality, but in most of the cases, if recovery takes place, complete restoration follows very soon. There are said to be children—I am quoting my informants—who get the disease every year just at the same time. These statements were somewhat astonishing and almost incredible; in handbooks and textbooks nothing could be found resembling this disease. Very soon I saw my first case, the only one during 1939 and this was already " cured " and the season over. It was the second attack of this child and I saw the blood-reports of its first attack in 1938 and the reports from the " interval." Neither the physician (an experienced haematologist), who saw the child a year ago, nor I myself could explain the clinical picture at that time. As had been forecast by the Baghdad physicians during the spring of 1940 the number of cases examined increased suddenly and from the end of March till the end of May fourteen cases came under exact observation.

*While this paper was in the press news was received that Professor LEDERER had died suddenly in Baghdad.

THE CLINICAL PICTURE.

This varies with the severity of the particular case. The children fall ill suddenly, the start of the illness can be given by the parents as at a definite hour. Usually abdominal pains and in most of the cases vomiting introduce the disease. The children become conspicuously pale at once, and after a few hours jaundice starts, though the children never become as yellow as in obstructive jaundice. The anaemia is in the foreground. Shortly after the first stage of pains and vomiting and the accompanying excitement the children become drowsy. They sleep most of the time. This is not a condition of complete loss of consciousness, but more a condition of continuous fainting. When roused they open their eyes but fall back into drowsiness at once. When examined or in any way stimulated, they start crying, but very soon the drowsiness returns. The anaemia increases very rapidly indeed, and in severe cases the condition assumes a dangerous appearance within 12 to 18 hours.

In examination actually nothing is to be found besides the anaemia and the jaundice. The general condition of the children is in all cases the same and will be described later. Temperature is normal in most of the cases. I noticed 39° C. only once. Pulse rate usually is very high ; only in slight cases was it about 120, in the majority up to 170 was counted. Respiration rate is only a little higher than normal. Enlargement of the spleen was found only three times. The organ was soft, not painful, not more than 1 to 2 fingers below the costal margin, and the involution of the rapidly enlarged organ could be noticed very soon in accordance with the quick recovery. Enlargement of the liver was found only twice. The urine was always passed in diminished amount, concentrated and of characteristic brown colour. The analysis never showed bile pigment or bile salts, only urobilin or urobilinogen in excess quantities. In four out of the fourteen cases albumin was found in traces : in only one very severe case a higher content of albumin, the presence of some granular and hyaline casts and haemoglobin could be seen. When recovery started the urine very rapidly became normal.

BLOOD REPORTS.

The interesting blood reports are in the foreground. Anaemia develops rapidly, only in the mildest cases were figures of about 3·5 million R.B.C.s found, usually the red cell count fell to one million per mm³. Hb. content was diminished on about the same scale, colour-index varying from 0·8 to 1·3. Anisocytosis and polychromasia were found in every case as well as normoblasts in varying amount. In severer cases megalocytes and megoloblasts were found.

The W.B.C.s showed an enormous increase, figures of 18,000 to 30,000 were found on the average, but also 45,000 and more were not uncommon. In all cases the neutrophils were increased, a remarkable fact considering the low age of all patients. Eosinophils were either absent or only up to 4 per cent.

Some myelocytes were found in almost every case. Platelets were diminished to half of the normal amount.

There are three blood reports in the following table showing the figures in a slight, an average and a severe case.

Fragility was determined several times and always found to be normal. Van den Bergh test was negative when examined in both phases.

The blood reports were all of the same character showing an acute haemolytic anaemia. The reaction of the bone marrow in the initial stage of the disease was very weak. The most striking fact is the rapid development of the anaemia. In the quoted case, Case 2, the illness began exactly at 4 p.m., the blood-examination was done at 7 a.m. the next morning, 15 hours after. The number of R.B.C.s dropped to 800,000 and the Hb. content to 12 per cent. Only in post-haemorrhagic anaemia can this rapid loss usually be seen.

Case No.	R.B.C. Millions.	Hb. per cent.	Colour Index	W.B.C.	Neutro- phils per cent.	Lympho- cytes per cent.	Eosino- phils per cent.	Baso- phils per cent.	Mono- cytes per cent.	Myelo- cytes per cent.	Plate- lets.
4	2,220,000	41	1	21,000	55	40	2	0	1	2	170,000
3	1,550,000	40	1·33	30,200	68	28·5	2·5	0	2	few	179,000
2	808,000	12	0·8	33,650	64	29	0	2	0	5	143,000

The other characteristic and striking fact is the seasonal incidence. This disease occurs only during spring, from about the middle of March to end of May. Strangely enough—there are children having the disease every spring. Case 3, e.g., whose birthday is on March 28th, has had the disease so far every year just at this time. Out of the fourteen cases one had the attack twice, two three times and one four times. During the year the children are perfectly well, but the parents know already that spring is a dangerous season for their children and fear it. The blood-picture does not become fully normal in all cases during the interval. Case 4, e.g., having during the attack 1,600,000 R.B.C.s and 40 per cent. Hb., 31,000 W.B.C.s, had 2½ months later 4,040,000 R.B.C.s, 63 per cent. Hb. and still 18,700 W.B.C.s. Case 5 had during the attack 1,160,000 R.B.C.s, 17 per cent. Hb., 11,700 W.B.C.s, one month later 4,280,000 R.B.C.s, 71 per cent. Hb., 6,900 W.B.C.s. A marked difference takes place in the distribution between neutrophils and lymphocytes. During the attack the neutrophils are prevalent, in the interval the lymphocytes come into the foreground again according to the low age of the children concerned.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS.

Diagnosis is not difficult. The clinical picture is so characteristic that confusion with other diseases is avoidable if the children are examined properly. Only one disease resembles this anaemia in its clinical appearance, the acute haemolytic anaemia type Lederer. But the cases of this disease published up till now concern older children, occur mostly after any one infection, and nothing is mentioned of any seasonal incidence or recurrence every year. The haematological picture is very similar.

Another disease having some similarity is the familiar haemolytic jaundice or, as it is better termed, familial haemolytic anaemia (Gaucher's disease). There are also attacks of acute anaemia with jaundice, not of obstructive character, occurring also repeatedly, sometimes with abdominal pains. But examination shows that this disease is quite different from the anaemia under discussion. The two main symptoms of Gaucher's disease, high fragility of the R.B.C.s and microcytosis, could never be found in our cases, on the contrary, fragility when examined (four times) was normal or low. The van den Bergh test, positive in Gaucher's disease was, in all our cases of anaemia examined, negative, and only once in the indirect phase positive. The characteristic spleen enlargement of Gaucher's disease also was absent in our cases. So differentiation of these two diseases is easy.

Another disease which could be confused with these cases of acute anaemia is blackwater fever. Here also differential diagnosis is not difficult. In none of the cases was there any history of malaria or administration of quinine. Oligury is mentioned in the severe cases but never anury. In all cases of blackwater fever high albumin content is a constant symptom and so haemoglobinuria is always seen, whilst only in one of our cases is albuminuria of higher degree and haemoglobinuria mentioned. In four cases traces of albumin could be found, the other nine were free of albumin.

COURSE OF THE ILLNESS.

The course of the disease is rapid in every case. The one fatal case died after 36 hours, and in the other three cases which had to be denoted as severe I had the impression that death would have occurred in the same short time if energetic treatment had not been started in time. In the cases which were cured and in the mild cases, recovery took place very soon, the shock symptoms ceasing after 1 day, the jaundice disappearing after 3 to 7 days, the anaemia after 2 weeks. As mentioned already blood reports were not always fully normal in the interval.

Prognosis.

Prognosis depends on the severity of the anaemia, the reaction of the bone marrow and the treatment. Both the kind of treatment and the time of its commencement are of importance. If the anaemia develops very rapidly, if

ed red cells are found, if the number of lymphocytes is low but of neutro-
igh, if only a few myelocytes are seen, the prognosis is bad. The other
which is of influence on the prognosis is proper treatment starting in
possible in the first 24 hours of the disease. I lost out of the fourteen
nly the first and this because I did not recognize the disease at that time.
he child 24 hours after the illness began and would not believe in the
of the case. The child died 12 hours later, before energetic treatment
en started. Later on three of the cases could be described as very severe
s below 1 million, Hb. from 12 to 21 per cent. after 15 to 24 hours of
), but none was fatal.

AETIOLOGY AND PATHOGENESIS.

he most interesting question of the origin of this disease has now to be
ed. I saw from the very beginning of these observations that the most
factor of seasonal incidence must give the key to a solution of the problem.
ow a number of diseases which are prevalent but not only during spring,
tance, certain forms of eczema, tetany, croup, asthma. (By spring we
eaking of " biological spring "). The incidence of these diseases is not
ctly bound to the season. They are only more frequent during spring
cur also during other seasons, and are not limited just to a few weeks in
ar. Another idea was that the disease might have some connection with
l seasonal spring, as for instance the blossoming of certain flowers. My
case was as severe as the first. I asked the parents whether the child,
2 years of age, had any contact with flowers. After some hesitation
rents and a servant confirmed that the boy had eaten, four to five days
, some flowers which he had collected in a public park. The same evening
wers concerned were brought to me. Mr. BOSWELL from the Biological
te identified them as *Verbena hybrida*, *Mathiola incana* and cornflower,
y common flowers blooming here during spring. My first searches
literature for similar diseases were without result, but finally I found that
ly and Sardinia a disease occurs showing very similar symptoms, the same
picture, very similar clinical findings and the same mortality. It is called
smo." This clinical picture was described first by MACCIOTTA (1926)
also shows acute anaemia, jaundice and a blood-picture similar to that
n our cases. The writer supposes the disease to be due to inhalation of
llen of beans or eating of fresh fruits. The symptoms start a short time,
36 hours, after the action of the exciting cause and show besides the symp-
of our cases, headache and diarrhoea. Whilst our disease occurs nearly
 in quite young children, in Sicily older children and adults also fall ill :
lity is 10 per cent. The author believes an anaphylactic shock to be the
More than 6 years elapsed before the next description of the disease
red in the Italian literature when MALLARDI (1933) from Naples described

a very severe case and then, as far as I can trace, four writers published similar cases (PIANA, 1933 ; BENTIVOGLIO, 1933 ; LEONE, 1935 ; CHIEFFI, 1935). The disease is said to have been spread over the whole Mediterranean in previous years, but occurs now only in Southern Italy, Sicily and Sardinia.   All authors agree that infection can be excluded, that intoxication or more likely anaphylactic sensitization is the cause.   Only one author could prove this last supposition by transmission of the anaphylaxis to rabbits by injection of the inactivated blood of a sick individual.

In an examination of our cases as to whether they had eaten beans (*Vicia flava*) or smelt or eaten other flowers we must neglect Case 1 because I did not know anything of this possible cause at that time.   In the subsequent thirteen cases I found this history of beans eleven times.   In one case it was strongly denied that beans had been eaten.   One case concerned a quite young child of 8 months which never had eaten any solids.   In the " positive " cases I found several times that the children had eaten boiled beans repeatedly and then raw ones immediately before the illness began—a point reminding us strongly of anaphylaxis.   It must be kept in mind that beans here are a very common food and eaten by almost everybody.

On the other hand five out of thirteen cases gave a clear history of near contact with strong smelling flowers and, as I have mentioned already, of having eaten these flowers, so that there is some exterior factor connected with blossoms and young fruits during spring seems to be certain.

It had now to be found out if intoxication or anaphylaxis plays a rôle. The former was not likely and anyhow the sudden onset and the general appearance reminded us of a poisoning.   I requested Dr. TOPOUS KHAN to keep some mice in a cage first with flowers of the kind concerned, then to take away their normal food and so to force them to eat these flowers, and finally to feed them with beans.   The animals thrived and did not show any signs of intoxication. So I abandoned this idea and started to bring a proof of anaphylaxis.   Unfortunately the season of our disease lasting only a few weeks was already well advanced and only a few experiments could be done.

I made contact with the Director of the Chemical Institute, Mr. HAWKINS, who gave me his most valuable help in preparing a protein-extract of a mixture of *Verbena*, *Matthiola* and cornflower on the one hand and fresh young beans on the other hand.   The crushed flowers and fruits were suspended in water, filtered, precipitated with ammonium sulphate by saturation, filtered, the precipitate washed with a little water, dried with acetone and dissolved in sterile distilled water.   A small amount of phenol was added and the extracts were kept in the refrigerator.

I first made with both extracts some intracutaneous injections on myself in order to be sure that no harm could be done by the injections.   Nothing happened and no reaction was seen.   Then I injected both the flower- and the bean-extract into three children all of whom had been admitted to the hospital for other diseases.   The bean extract injected into three children did not give

any reaction. The flower extract showed in two children a small redness of scarcely 1 cm. diameter, the third child did not give any reaction. Then I had an opportunity of administering the extracts to children with this anaemia. Two children injected with bean extract did not show any reaction, but two children injected with flowers extract showed after 24 hours a big redness of 3 to 4 cm. diameter ; in one case there was even a little infiltration as in a positive Mantoux test. One of these " flower-positive " children was negative to bean extract. I am giving these results with every reservation, the time was too short and the number of children tested too small to draw definite conclusions. The difference between bean- and flowers-extract may be due either to real difference in sensibility to the two " antigens " or may be explainable by the fact that the bean extract was dried more with acetone and was not as stable as the flowers extract which I kept safely in the refrigerator for several weeks. A new proof on a larger number of cases, especially a differentiation between the particular flowers in the mixed extract, further the Prausnitz-Küstner experiment, the transmission of the anaphylaxis to another individual must be held over for the next year. That this kind of anaemia is due to anaphylaxis seems to be proved by the observations mentioned above. The few experiments done up to now seem to be in favour of the flowers being the cause.

The objection can be made that thousands of children here in Baghdad are in contact with these very common flowers as well as with beans which are a very popular food. Why are only a small number of children liable to get this disease ? Do individual differences play a role in this respect ? This question can be answered in the affirmative. There are five factors which mark those children liable to this disease. First of all the climate. The disease occurs only in the hot climate and is therefore similar to " Favismo " and sickle cell anaemia which are both encountered in Mediterranean countries. Secondly the age. The oldest child I saw was $4\frac{1}{2}$ years of age, the youngest only 8 months. " Favismo " occurs at every age. Thirdly, the sex. All our cases were boys, a factor which is mentioned in no similar disease. Fourthly, the race. Thirteen of the fourteen cases were Jews. Only one Mohamedan child fell ill. Finally, the individual constitution ; all the children without exception were well nourished, rather fat and pasty, of a type we call the " Rubens-type," the younger ones all with symptoms of rickets, and all of light complexion. Not one of the children had black hair and dark skin, six had blond, seven brown and one red hair, usually curly. These five factors are so significant that they cannot be overlooked, and it seems certain that individual factors influence the incidence of the disease.

So the circle seems to be closed : This form of acute anaemia is a well-characterized disease due to contact with flowers or fruits only during spring. The observations lead us to suppose that an anaphylactic sensitization is the cause, with incorrect or deficient reaction of the bone marrow, occurring only in individuals living in a hot climate and showing significant characteristics of their individual constitution.

Treatment

As mentioned repeatedly the treatment gives very gratifying results if started in time and done energetically. Two factors have to be considered if successful treatment is to be carried out, namely the anaphylactic shock and the inactivity of the erythropoietic system with its rapid development of severe anaemia. Three therapeutic measures have been proved to be most successful, (1) administration of blood, (2) liver treatment in high dosage and (3) injection of adrenalin. Blood administration acts in both directions, against the shock as well as stimulant to the bone marrow. In mild cases whole blood injections, 10 to 20 c.c. i.m. is sufficient, but in severe cases I strongly advise transfusion. In Case 2, I transfused 150 c.c. blood and the effect was striking. In Case 11 the transfusion was refused and whole blood injections had to be given throughout 5 days, the child being all the time in a rather dangerous condition. It finally recovered after having received 100 c.c. whole blood i.m. With transfusion much time and heart strain could have been spared. Liver should be injected in high dosage, every day or on alternate days one ampoule of the usual preparations. Adrenalin should be given from the very beginning. I chose it because its action in every case of shock is well known and it is a stimulant to the bone marrow at the same time. The results seemed to me to be much more satisfactory and rapid since I introduced this therapy.

I am grateful to all physicians who sent me cases for observation and who kept in touch with me all the time. My thanks are especially due to Mr. HAWKINS, Dr. TOPOUS KHAN and Mr. BOSWELL who assisted me with their valuable aid, both in advice and co-operation. As this disease was first described here and was cleared up by the co-operation of so many local personalities I suggest that it should be called "Baghdad spring anaemia."

SUMMARY.

A new form of acute anaemia is reported occurring in Baghdad only during a few weeks of Spring. The disease runs a very rapid course, develops anaemia within 24 to 36 hours, has a mortality of about 10 per cent. and is curable by injection or transfusion of blood, liver therapy in large dosage and adrenalin. Only boys, most of them being Jews, of a significant constitutional type are affected. This anaemia is due to anaphylaxis by contact with flowers and young fruits.

The name "Baghdad spring anaemia" is suggested for this disease.

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BUSH-TEA HAEMATURIA.

BY

K. VIGORS EARLE, M.D. (LOND.), B.CH. (CANTAB.).*
*Second Government Medical Officer, Administration of Nauru,
Nauru Island, Central Pacific.*
(*late Medical Officer, United British Oilfields of Trinidad, Ltd., Trinidad,
British West Indies.*)

Pre-eminent as home remedies in certain West Indian islands (*e.g.,* Barbados, Trinidad, Tobago and Martinique). are home-brewed " teas " or infusions of roots, plants, bushes and parts of trees. The list of such plants having an alleged medicinal value is beyond the scope of this paper, which deals with certain pathological effects which may follow the misuse of such infusions.

Two plants are used specifically in the treatment of gonorrhoea, " rheumatism ", and to procure abortion (and empirically in almost any other condition !), namely, " gully-root " (Barbados) and lignum vitae (most West Indian islands). Both these plants, if used to excess, or possibly in susceptible subjects) may produce a violent haematuria. Only the lignum vitae will be dealt with in this paper.

LIGNUM VITAE.

Guaiacum officinale Linn. (Family : Zygophyllaceae.) Indigenous to the West Indies and the north coast of South America. In Barbados its usual habitat is near residences in country districts. It is a medium-sized evergreen tree, with wide-spreading, stout branches. It commences flowering in April, the flowers being madonna-blue in colour, numerous and measuring an inch

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As mentioned repeatedly the treatment gives very gratifying results if started in time and done energetically. Two factors have to be considered if successful treatment is to be carried out, namely the anaphylactic shock and the inactivity of the erythropoietic system with its rapid development of severe anaemia. Three therapeutic measures have been proved to be most successful, (1) administration of blood, (2) liver treatment in high dosage and (3) injection of adrenalin. Blood administration acts in both directions, against the shock as well as stimulant to the bone marrow. In mild cases whole blood injections, 10 to 20 c.c. i.m. is sufficient, but in severe cases I strongly advise transfusion. In Case 2, I transfused 150 c.c. blood and the effect was striking. In Case 11 the transfusion was refused and whole blood injections had to be given throughout 5 days, the child being all the time in a rather dangerous condition. It finally recovered after having received 100 c.c. whole blood i.m. With transfusion much time and heart strain could have been spared. Liver should be injected in high dosage, every day or on alternate days one ampoule of the usual preparations. Adrenalin should be given from the very beginning. I chose it because its action in every case of shock is well known and it is a stimulant to the bone marrow at the same time. The results seemed to me to be much more satisfactory and rapid since I introduced this therapy.

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across. The fruit is bright orange in colour. The bark is thick and covered with a greenish crust which readily peels off. The wood is dense (heavier than water), hard and greenish-brown ; from it exudes a gum which has well-known medicinal properties.

Before dealing with the uses of lignum vitae in native medicine, the active constituents present in the wood will be rapidly reviewed. These are described in detail in the *British Pharmaceutical Codex* (1934). The wood contains guaiacum resin in which the following acids are found :—α and β guaiaconic acids (about 70 per cent.), guaiaretic acid (about 11 per cent.) and a small proportion of guaiacic acid. It also contains guaiac-resin (about 15 per cent.), and small quantities of guaiac yellow, vanillin and guaiac saponin. Guaiacum resin is stated to be a mild laxative and diuretic and it has a place in the therapy of chronic rheumatism and gout and in dysmenorrhoea.

USES IN NATIVE MEDICINE.

In native medicine any part of the plant may be used—leaves, bark or wood. The selected parts may be boiled and the resulting infusion taken hot, or bottled and taken in doses, or boiling water, may be poured over them and the supernatant fluid taken in a variety of ways.

Such haphazard methods of preparation result in " teas," the pharmacological actions of which are quite unpredictable. For example, it is not known whether the leaves contain all of the constituents listed above or whether crude methods of preparation result in a preponderance of one or more constituents.

The dosage of the tea depends on the taste of the individual—massive, or in given quantities at stated intervals. It may also be taken (secretly) together with drugs or injections prescribed by a qualified European practitioner.

In gonorrhoea the tea is employed in acute and chronic urethritis and also in myositis, synovitis and arthritis of gonococcal origin. Conditions loosely referred to as " rheumatism" may also be treated by this method. As an abortifacient bush-tea enjoys a wide popularity, though its action, like many drugs used for a similar purpose, is quite uncertain.

Toxic reactions.

These appear to originate in three different ways :—
  (1) Following a single massive draught of the tea.
  (2) Following repeated small doses.
  (3) In susceptible persons quite minimal doses, which would be without effect in normal individuals, can produce toxic manifestations.

According to BAYLEY (1939) haematuria usually sets in 3 to 4 days after taking the tea, but in my experience it may occur within 48 hours of ingestion of the drug. The patient may complain of backache, located in the lumbar

region, possibly slight chills and rigors and slight malaise. His principal and often only complaint, however, is of discoloration of the urine. This may range from a slight tinging with blood to a black urine similar to that seen in blackwater fever.

On examination the urine may show the range of colour change mentioned above; it is usually acid in reaction but the specific gravity rarely exceeds the normal limits of 1,015-1,025. Granular and epithelial casts are present in varying quantities, whilst red blood-cells are present, usually in large numbers. Tests for haemoglobin are positive, but I suspect that other blood-pigments are also present: these I have had no means to demonstrate.

Observations on the toxic reactions.—Where a single massive dose or repeated small doses have been taken the reaction is obviously due to one or more of the constituents listed above—which one it is impossible to say since varying methods of preparation may result in the elimination of one or more.

There are, however, cases where the dosage of the tea has been extremely small yet a well-marked haematuria has occurred—these are cases of individual idiosyncrasy. Such idiosyncrasies to plants, producing haematuria and haemoglobinuria among other symptoms, are by no means uncommon. Haemoglobinuria following ingestion of cranberries (*Vaccinium uliginosum*) has been described by FRENKIEL (1932). " Favismo " (MACCIOTTA, 1926) or " Bohnenkrankheit " (FINKELSTEIN, ? date) are symptom-complexes following ingestion or inhalation of the seed, plant or pollen of the common (Windsor) bean. "Favismo," which occurs in Sicily, Sardinia and southern Italy is characterized (in mild cases) by headache, dizziness, slight mental confusion and sometimes by vomiting and diarrhoea. Severe cases display haemoglobinuria, icterus and anaemia; liver and spleen are enlarged, the temperature is raised and a pronounced leucocytosis occurs. In such severe cases the mortality-rate may amount to 10 per cent.

LOTTI and di MANAI (? date) have produced haemoglobinuria in rabbits, prevously sensitized with bean-extract, by further injections of bean-extract. Because of these findings most Italian writers regard "Favismo" as an anaphylactic phenomenon.

It is likely, therefore, that the urinary symptoms which follow the ingestion of small amounts of lignum vitae are also anaphylactic in nature.

Treatment.

The patient should be put to bed and given a fluid, non-protein diet. Barley-water or coconut water may be given and mineral waters (*e.g.*, Vichy, Contrexéville) may be allowed. A mixture containing sodium bicarbonate and potassium citrate should be prescribed. Kaolin poultices or thermogene wool should be used for lumbar pain. Cure is usually rapid, generally occurring within 2 days. I have never seen a fatal case.

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Typical case history.

B. N., aged 23, negro (Barbadian), labourer. First seen on April 8, 1937, when he complained of backache and passing blood-stained urine. He gave a history of a fresh attack of gonorrhoea, the discharge having first appeared four days ago. Two days before being seen he took a massive draught of bush-tea, prepared by boiling lignum vitae leaves. Twenty-four hours later he felt backache which was followed a few hours later by a mild haematuria which increased in intensity.

Examination showed a robust young man with no lesions in cardio-vascular, alimentary, pulmonary or central nervous systems. There was tenderness on deep pressure over the renal area. A urethral discharge, containing gonococci, was present. No malarial parasites were found in the blood-film.

The urine was port-wine in colour, slightly acid in reaction and with a specific gravity of 1,020. The centrifuged deposit showed red blood-cells and granular and epithelial casts. Spectroscopic examination was not performed.

Treatment consisted in putting the patient to bed, giving an alkaline mixture together with large amounts of fluid (especially coconut water). The haematuria ceased within 24 hours and the patient made an uninterrupted recovery.

SUMMARY.

1. An account of the lignum vitae is given.

2. Its uses in native medicine are described.

3. Toxic and anaphylactic reactions following its use are indicated.

4. Treatment of these conditions is outlined.

5. A typical case-history is appended.

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TREATMENT OF LARVA MIGRANS

BY

H. H. BAYLEY, M.A., M.B., B.Ch. (CANTAB.),
St. Michael, Barbados, British West Indies.

Larva migrans is a persistent and troublesome condition which is found in the epidermis. It is produced by the larvae of some species of nematode, chiefly those of Ancylostoma braziliense, Gastrophilus haemorrhalis and G. veterinus.

In Barbados the infection is frequently found in children and adults who have handled the sand above high-water mark and MANSON-BAHR* records this as the origin of the infection in Florida. Certainly great quantities of flies inhabit the beaches here, feeding on the rotting seaweed and fish which is plentiful.

The lesion is characterised by a raised zig-zag line approximately 2 mm. broad: every 24 hours there is an increase in length of about $2\frac{1}{2}$ cm. At first it resembles an insect bite in appearance and in the intense itching which accompanies it; when seen at this stage the diagnosis is difficult; the larva may remain localized for some time but as soon as it has started to migrate the typical burrow is plainly seen with the naked eye.

* Manson's Tropical Diseases (1940), 11th Ed., p. 853. London : Cassell & Co.

The usual treatment has been the destruction of the parasite by various chemical and physical agents. On the whole they have not been satisfactory and the infection usually remains to annoy the patient for many months. The following method of treatment has been tried and found successful: all cases so far treated having been entirely cured within a few days. LOMBARD has shown that the human epidermis can be easily made transparent and it is by this method of clearing the skin that the larva is located.

Treatment.

The skin is thoroughly dried and cleaned with alcohol. Cedar wood oil is used to clear the affected area which is then examined with the $\frac{2}{3}$ inch objective of a dissecting microscope. A good hand lens can also be used with advantage.

The ends of the burrow must be carefully examined as it is here that the larva may usually be found. It stands out clearly as a white, spherical mass due to the presence of plasma which surrounds it. Having noted the position of the larva the skin is cleaned for operation and 2 minims of procaine 1:1,000 is used to desensitize an area half an inch in diameter with the larva in the centre. A cautery is then applied until a small burn is produced. Sulphanilamide, grains 7½, is prescribed for two days: this helps the burrow to heal, as it has obviously been contaminated by bacteria and much of the itching is caused by this secondary infection. When the patient returns in three days' time a faint brown line and a small cautery burn are all that remain and no further treatment is necessary.

Each burrow must be examined and wherever a larva is discovered the cautery has to be applied.

SUMMARY.

A short description of infection with larva migrans.
Discovery of the larva by clearing the skin with oil and examination with a dissecting microscope.
Treatment by the use of a cautery and sulphanilamide.

POLYSACCHARIDE SCOLEX ANTIGEN FOR THE IMMUNOLOGICAL DIAGNOSIS OF HYDATID DISEASE.

BY

H. A. SENEKJI, M.D.
*Department of Bacteriology,
Royal College of Medicine, Baghdad, Iraq.*

For the clinical diagnosis of hydatid disease in man, it is important to have a stable standard antigen. The antigen which has been most commonly used for the detection of precipitins, complement fixing antibodies and specific allergy in man and animals suffering from hydatid disease has been the bacteriologically sterile hydatid cyst fluid aspirated from fresh fertile hydatid cysts of sheep and cattle. N. H. FAIRLEY (1922) demonstrated that the fluid from human sources has little or no antigenic properties.

K. D. FAIRLEY (1923) showed that the fluid obtained from fresh fertile hydatid cysts of sheep to which was added phenol in $\frac{1}{2}$ per cent. concentration gave 65 per cent. positive results. N. H. FAIRLEY (1922) followed the lead of WEINBERG (1909) in applying the complement fixation test to the serological diagnosis of hydatid disease. He found that an alcoholic extract of the scolices gave good results, but as its preparation was somewhat difficult, he preferred the hydatid fluid antigen without any preservative for the complement fixation and precipitin tests.

Recently, DENNIS (1937) used the trichloracetic acid precipitate of the fluid from the fertile hydatid cysts. For the precipitin tests, he recommends three different concentrations of the purified antigen, namely, 1 : 1,000, 1 : 10,000 and 1 : 50,000 using a constant volume of antiserum. For the complement fixation test, he recommends 1 : 5,000 concentration, while for the Casoni intradermal test for specific allergy he uses 1 : 10,000 concentration. The antigen of Dennis is the purified hydatid protein of the hydatid fluid.

The purpose of the present paper is to describe an antigen, apparently of carbohydrate nature, prepared from scolices, and which has been found to give good results in the laboratory diagnosis of hydatid disease.

MATERIAL AND METHODS.

The scolices are collected under aseptic conditions from the fresh fertile cysts from the liver and lung of sheep and cattle. They are then separated from the debris by centrifuging at a low speed, washed three times in saline, and finally resuspended in saline to which formalin is added in 0·1 per cent. concentration, and stored in the ice chest.

The scolices· of the different lots are pooled together and extracted in four volumes of pure acetone at 37° C. overnight. The sediment is collected by centrifuging, dried at 37° in the incubator, ground up in a clean mortar, weighed and stored in a CaCl$_2$ desiccator in the dark.

Three grammes of the powdered scolex material are then extracted in 750 c.c. of N/4 trichloracetic acid for 48 hours in the ice chest. The supernatant, which is very opalescent, is separated by centrifuging. On neutralizing the supernatant with 40 per cent. NaOH, a cloudy cotton-wool precipitate is immediately formed ; the flask is then returned to the ice chest and kept overnight. The precipitate is collected by centrifuging, washed twice in absolute· alcohol, once in ether, dried at 37° C. in the incubator, ground up into a fine powder in a mortar, weighed and stored in a desiccator in the dark. The yield is about 5 per cent. of the dried scolex. This powder retains its antigenic potency for a long time in the dark in the desiccator.

PHYSICAL AND CHEMICAL PROPERTIES OF THE ANTIGEN.

The extract is soluble in acids, but in alkaline solutions it immediately precipitates. It does not react to the biuret and Millon's tests for the proteins, but gives a very strong reaction to a naphthol Molisch test for carbohydrates. It does not reduce the Benedict's reagent, but on hydrolysis reduces the reagent. The phenylhydrazine test is negative for the osazones.

Preparation of the solution for use in the tests :—25 mg. of the powder are dissolved in a small amount of N/1 HCl. The reaction is brought to neutrality by N/1 NaHCO$_3$ and the solution is made up to 10 c.c. The resulting solution is somewhat colloidal and therefore cannot be filtered through Seitz or porcelain filters. Consequently it is necessary to carry on all this procedure under strictly aseptic conditions. Sterility tests are made by aerobic and anaerobic methods. For the Casoni test, phenol is added in $\frac{1}{2}$ per cent. concentration, while for the precipitin tests no preservative is added. The antigen in this state keeps for 2 months provided it is kept in the ice chest.

Immunological Studies.

The specific allergy test of Casoni is performed by injecting 0·25 mg. of the antigen which is contained in 0·1 c.c. The positive reaction appears immediately, and does not differ from that of the skin test where the hydatid fluid is injected. We have tested 100 patients suspected of hydatid disease with the hydatid fluid and polysaccharide fraction. In all these cases there was complete correlation between the two antigens. The precipitin tests are carried out with 1 : 400 and 1 : 4,000 concentrations of the polysaccharide using a constant volume of the antiserum. In our experience the precipitin tests have not given very satisfactory and uniform results.

DISCUSSION.

Since the human hydatid fluid had been shown to have poor antigenic properties, it cannot be used for the laboratory diagnosis of hydatid disease. Sheep or cattle hydatid fluid is antigenically very potent. There may be considerable variation between the potency of one batch and that of another. The scolex antigen can be prepared in large amounts, and its keeping potency is good, especially in the powder form. It is a polysaccharide and is free of proteins. It is not only a haptine but is also a functional antigen. In the routine clinical laboratory diagnosis of hydatid disease by the specific allergy test of Casoni, it gives uniform and reliable results, because a constant uniform quantity of the antigen is used throughout the tests.

SUMMARY AND CONCLUSIONS.

1. A specific polysaccharide is extracted from hydatid scolices by N/4 trichloracetic acid.

2. For the specific allergy test, 0·25 mg. of the antigen is recommended, and for the precipitin tests 1 : 400 and 1 : 4,000 concentrations.

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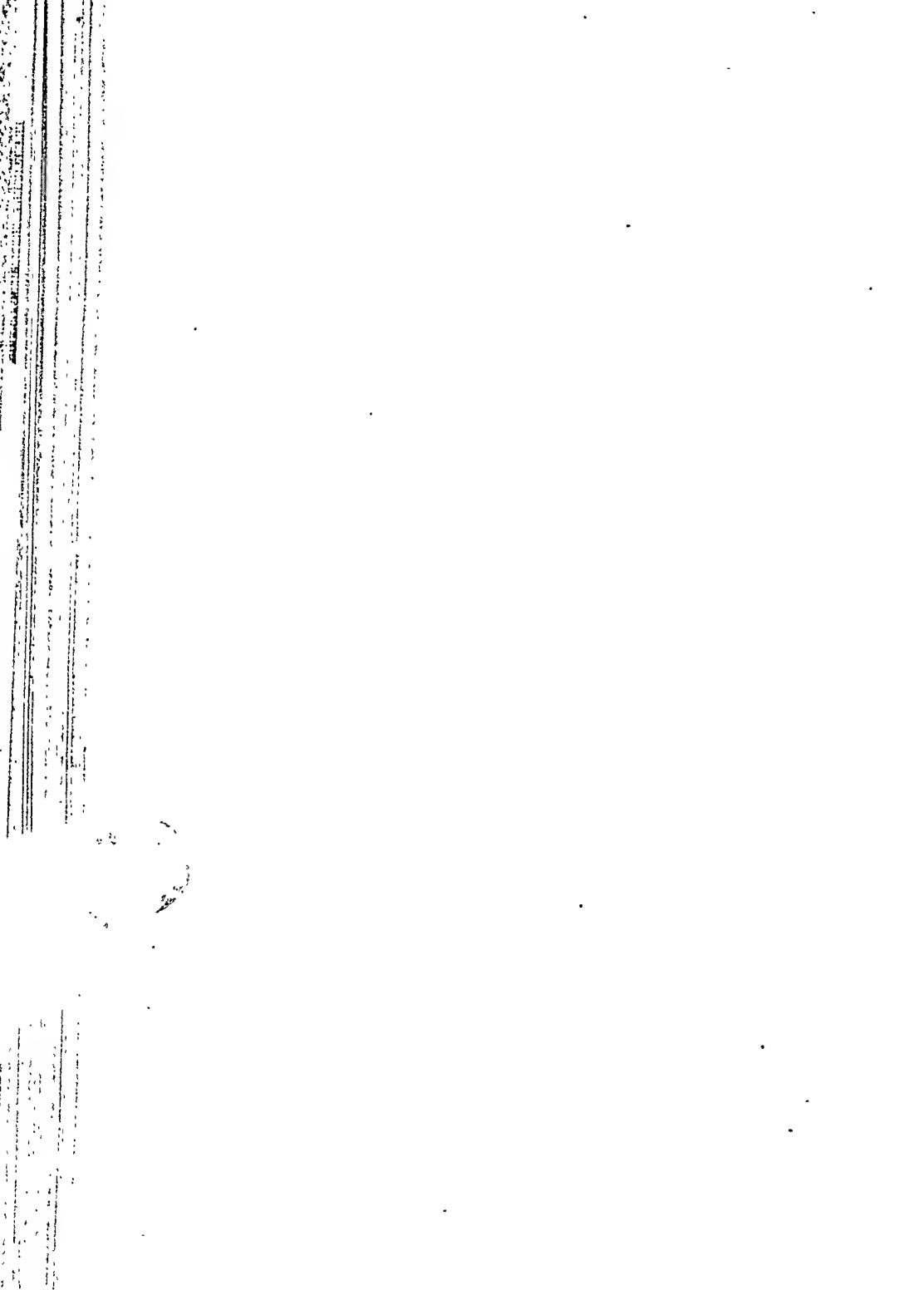
COMMUNICATIONS.*

THE MORPHOLOGY OF MALARIAL PARASITES IN THICK BLOOD FILMS.

PART IV.
THE IDENTIFICATION OF SPECIES AND PHASE.

BY

J. W. FIELD, M.D.

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The malarial parasite in fixed thin blood films is confined within the host red cell; in lysed thick films the parasite lies free in the plasma. To this fact is related much of the difficulty of the thick-film diagnosis of malaria. Diagnosis from thin films is based not only on the appearance of the parasites themselves but also on changes occurring in the host cells—the enlargement of the cells containing *Plasmodium vivax*, the distinctive appearance of Schüffner's, Maurer's and Ziemann's dots, the occasional cremation and darkening of the rim of cells infected with *P. falciparum*; these and other changes always aid diagnosis and may alone sometimes be sufficient to enable the species to be identified. The blood in thick films, however, is lysed, the red cells are not visible and the recognition of species must be made from criteria other than the changes in infected cells. Moreover, the parasites themselves may be changed in appearance; they are exposed to physical stresses which are inherent in the thick-film staining process and are often damaged; their outlines are subjected to erosion and they tend therefore to appear somewhat ragged in outline and reduced in size.

The recognition of parasites and the identification of species in thick blood films might well seem from these disturbing influences to be peculiarly difficult,

* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

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but there are two great compensating advantages—concentration of parasites and an improved definition of pigment. The numerical advantage over thin films of a fifteen- to twenty-fold increase in the number of parasites seen in a given time or in a given number of microscopic fields is a very great diagnostic aid, and species diagnosis may often be made from the general parasite picture where individual parasites are not specifically distinctive. Furthermore, the pigment tends to be seen more clearly in thick films and the parasite species may sometimes be identified solely from its form and distribution. In thin films the pigment of all forms excepting mature schizonts is confined within the blue-stained cytoplasm and often is visible only as a vague greenish blur; moreover the granules may be intermingled with a confusing profusion of dots from the stippled host cells; but in thick films the infected cells have disappeared, there is little or no stippling, the cytoplasm itself is less dense in texture and the pigment granules shine through with greater clarity.

Specific diagnosis from thick blood films is not, as a rule, difficult except when infections are light. Species and phase may normally be recognised as readily as in thin films and in a tenth of the time, but the diagnostic criteria are different. The differences are discussed in this paper. The general principles of species diagnosis from thick blood films are outlined and an attempt is made to define the limits of diagnostic accuracy of the thick-film method. (See Plates XIII to XVII at end of paper.)

1. Specific Differences in Thick Film Morphology.

For descriptive convenience the morphological differences of *P. falciparum*, *P. vivax* and *P. malariae* are considered at each of the following growth phases —young unpigmented trophozoites, older pigmented trophozoites, schizonts and gametocytes.

(a) Young Unpigmented Trophozoites.*

The morphological differences in thick blood films between individual trophozoites of the three common species are very small and diagnosis from isolated parasites is often difficult and may be impossible. The trophozoites of *P. falciparum* are usually smaller than those of other species—though occasionally they are fully as large as those of *P. vivax*—and the cytoplasm, though varied in pattern, is relatively compact; *P. vivax* tends to larger size and to greater irregularity; *P. malariae* often has a larger chromatin bead in relation to the amount of cytoplasm than have the other species.

The most important diagnostic aid lies, however, not in the morphology of individual trophozoites but in the composite microscopic picture—in the number of trophozoites present and in the stage of development of the associated forms. The greatest numbers are found with *P. falciparum*, the smallest with *P. malariae*. *Falciparum* infections may show more than 100 parasites per microscopic field; quartan infections rarely more than half a dozen. Heavy

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* The term "young trophozoite" is perhaps preferable to "ring form"; young unpigmented parasites in thick films may have little resemblance to rings.

infections in which young trophozoites of a uniform sta[ge]
present are almost invariably due to *P. falciparum.* Th[e]
marized in Table I, made in 3,000 cases of untreated [malaria in a]
hospital associated with this laboratory, illustrate th[e]
difference in the degree of infestation.

TABLE I.

Trophozoite Concentration in the Peripheral Blood in [...]

Species.	Cases observed.	Trophozoites per c.mm. of [...]		
		Under 5,000.	5,000– 25,000.	25,000– 100,000.
		Per cent.	Per cent.	Per cent.
P. falciparum	2,037	20	42	31
P. vivax ...	896	37	51	11
P. malariae	67	73	27	<1

More important, however, than the number of troph[ozoites]
differences in their associations with other forms. *P. f[alciparum]*
tendency to invade the peripheral blood only at an ear[ly]
cycle and in the later stages of gametogony. The limite[d]
sented in the peripheral blood is extended only with inte[nse]
later stages of asexual growth and the early stages of [...]
also be seen. Thus, in most cases of *falciparum* malar[ia]
in thick films are young trophozoites and/or fairly matu[re]
tendencies are summarized in Table II.

Young *vivax* trophozoites, when present in numb[er]
fever, are usually accompanied by more distinctive form[s]
schizonts or/and gametocytes. *Vivax* infections in wh[ich]
parasites develops with perfect synchronism are rare [in]
district of the Malay States and, with parasites in sig[nificant]
seldom that species diagnosis must needs be based on yo[ung]

P. malariae has a greater tendency to synchron[ism]
P. vivax; but, even so, perfect synchronism, with all th[e]
the same stage of development, is rare; and, even wi[th]
young trophozoites are commonly accompanied by for[ms]

TABLE II.

Forms of *P. falciparum* in Thick Films from the Peripheral Blood.

	Young tropho-zoites.	Older tropho-zoites.	Schizonts.	Young spindle or lanceolate gametocytes.	Mature gameto-cytes.
Mild or moderate infections :					
(a) early	+	0	0	0	0
(b) late untreated ...	+	0	0	0	+
(c) quinine treated ...	0	0	0	0	±
Heavy infections :					
(a) early	+++	0 to ±	0 to ±	0 to ±	0
(b) late untreated ...	+++	0 to +	0 to +	0	+
(c) quinine treated ...	0	0	0	0	+

It is to be noted furthermore that the unpigmented trophozoite stage of *P. malariae* in the peripheral blood is brief—probably not more than a quarter of the schizogony cycle of 72 hours—and that only in a quarter or less of quartan films examined in the course of diagnostic routine is the young trophozoite the dominant form.

(b) OLDER TROPHOZOITES WITH COMMENCING PIGMENTATION.

The species differentiation of pigmented trophozoites is seldom difficult even with light infection. *Falciparum* trophozoites at this stage are nearly always associated with, and usually greatly outnumbered by, younger unpigmented forms. They are small and relatively compact; a single bead of chromatin and a dull blur of pigment may, or may not, be visible depending on the depth of staining. They are seldom seen in the peripheral blood except with heavy infections. Occasionally the start of quinine treatment seems to stimulate their appearance.

. *Vivax* trophozoites at the same stage of growth are larger, more delicate in texture, more irregular in outline, not necessarily associated with younger forms and often accompanied by schizonts and/or gametocytes. The cytoplasm has a marked tendency to scatter into delicate wisps and strands; often it breaks up into a cluster of fragments irregularly dispersed around the chromatin —a feature which I believe to be specifically distinctive. Pigment, in the form of small granules and rodlets, is scattered irregularly in the cytoplasm or isolated nearby.

The tendency for the cytoplasm of *P. vivax* to disperse at this stage is in marked contrast to the compact solidity of *P. malariae*. Contraction, closure of the vacuole and the assumption of a close-knit rounded form and texture occur very early in growth. This general form is retained for most of the

asexual cycle*. The pigment is profuse and is in the form of fairly coarse granules scattered throughout the parasite and often masking the chromatin. Pigmented trophozoites may be associated with young unpigmented trophozoites, schizonts or gametocytes but are often unaccompanied by other forms.

(c) SCHIZONTS.

Specific diagnosis at this stage is based on the size, the number of merozoites, the appearance of the pigment and the association with other forms.

Falciparum schizonts are comparatively rare in thick blood films. They are especially associated with heavy infestation and are always accompanied by large numbers of young trophozoites. The schizonts of *P. vivax* and of *P. malariae* are common in the peripheral blood and have no such distinctive tendencies in their associations with other forms. The frequency with which *falciparum* and *vivax* schizonts are found in thick blood films is indicated in Table III based on films examined from 2,965 cases of acute untreated malaria.

TABLE III.

Frequency of Schizonts in Thick Blood Films from 2,965 cases of Acute Untreated Malaria.*

Species.	Cases.	Evidence of schizogony in blood films.	Schizont incidence.
P. falciparum	2,062	62	3 per cent.
P. vivax ...	903	533	59 ..

* With *P. malariae* early schizogony is often difficult to recognize in thick blood films ; hence the omission of this species from Table III.

The tendency for *falciparum* schizonts to appear in the peripheral blood only when infection is intense is shown in Table IV, which summarizes the parasite count in the sixty-two cases in which schizonts were found. (See Table III.)

The size and form of the schizonts, the number of merozoites and the form of the pigment are also useful aids to diagnosis. *Falciparum* schizonts are relatively small, the merozoites are more numerous than in other species—up to twenty-five or more—and are usually arranged in a fairly close cluster ; the pigment is a single solid-looking dark mass. The schizonts of *P. vivax* are relatively larger, the merozoites seldom number more than sixteen, and the pigment is looser and lighter in colour. Schizonts of *P. malariae* are small,

*KNOWLES (1930) and YOUNG (1940) have worked out an approximate growth time table for *P. malariae* : their figures show that the trophozoite stage may persist for about two-thirds of the asexual cycle of 72 hours. Their calculations are as follows :—

KNOWLES and DAS GUPTA (1930)		YOUNG, STUBBS and COATNEY (1940)	
Free merozoites ...	1 hour.	Trophozoites	54·2 hours
Rings	17 hours.	Young schizonts (2 to 5	
Growing trophozoites ...	47 ,,	chromatin masses) ...	10·4 ,,
Schizonts	6 ,.	Segmenters (6 or more	
Mature rosettes ...	1 ..	chromatin masses) ...	7·2 ,,

TABLE IV.

Parasite Counts in sixty-two cases of Acute Untreated *falciparum* Malaria in which Schizonts
were found in Thick Blood Films.

Cases in which Schizonts were found.		Parasite count per c.mm. of blood.		
Non-fatal.	Fatal.	Less than 100,000.	100,000–500,000.	Over 500,000.
47	15	26	28	8

the merozoites are prone to scatter and seldom number more than eight ; the
pigment has a tendency to concentrate but is sometimes dispersed.

Difficulties arise mainly with immature schizonts. Half-grown *vivax*
schizonts with less than eight chromatin segments may closely resemble
malariae ; differentiation may then be possible only from the pigment and the
associated forms present.

(d) GAMETOCYTES.

The identification and the species differentiation of gametocytes are not
as a rule difficult. The distinction between gametocytes and the asexual forms
depends on the size, the shape, the absence of chromatin division and the
distribution of the pigment ; species differentiation depends on the size, the
shape, the pigment and the association with other specifically distinctive forms.

Mature *falciparum* gametocytes which retain their " crescentic " or
" sausage " shape are unmistakable but, with contraction from maturation
changes first to an ovoid then to a rounded form, they are progressively more
difficult to identify. Rounded *falciparum* gametocytes bear a close resemblance
to *P. malariae* : they are distinguished mainly by differences in pigment—
coarse rodlets with *falciparum*, fairly coarse granules with *malariae*—and by the
differences in the associated forms. The former are likely to be associated
with other gametocytes still retaining their elongated shape and with young
trophozoites of a uniform stage of growth ; the latter with other developmental
forms of *P. malariae*. Immature *falciparum* gametocytes, with their elongated
shape, their pointed ends and their scattered rodlets of pigment, bear no
resemblance to any other form of malarial parasite seen in the peripheral blood
and are readily identified.

Vivax gametocytes are recognised by their size, their undivided chromatin,
their scattered rodlets and granules of relatively fine pigment, a greater
tendency to disruption than is usual with other species, and their association
with other specifically distinctive forms. The pigment tends to cover a greater
area than that of any other malarial parasite and identification is sometimes
possible from this wide spread of fine pigment even when the chromatin and
cytoplasm are lost. It is seldom, however, that species diagnosis rests on the
recognition of an isolated gametocyte ; except in very light infections other
forms are likely to be found.

Quartan gametocytes are perhaps the most difficult of all malarial parasites to identify with precision in thick blood films. They are obviously malarial parasites; they can probably be recognised as quartan parasites, but they are often indistinguishable from large quartan trophozoites. They have a general resemblance to rounded *falciparum* gametocytes from which they are distinguished by the differences in the pigment and in the associated forms which are likely also to be present. From *vivax* gametocytes they differ in the smaller size, the more compact form and the coarser, more profuse pigmentation. But from large trophozoites of their own species there is often no satisfactory morphological distinction other than the tendency for the gametocyte to be of somewhat larger size, for the cytoplasm to be more uniform in texture and for the chromatin to stand out with somewhat greater prominence. Even so, on several occasions the writer has seen what appeared to be numerous quartan gametocytes in a thick blood film, and found a few hours later that most of them were in early schizogony.

2. MIXED INFECTIONS IN THICK BLOOD FILMS.

Many, perhaps most, of the errors which an inexperienced worker is likely to make in the thick film diagnosis of malaria are likely to be due to the failure to recognise more than one species of parasite in mixed infections. The identification of mixed species may be easy; it may be difficult or even impossible. The dominant species is usually evident from the general picture; the secondary species, if in small numbers, can be identified only if the forms present are specifically distinctive. The possible combinations of species and phase are very numerous and it is on the particular combination and, of course, on the numbers available for examination that the ease or difficulty of diagnosis depends. Examples of such combinations are given below for illustration :—

(*a*) As a rule easy to identify both species :

Dominant.	Secondary.
i. *Falciparum* trophozoites	*Vivax* at any stage beyond the young trophozoite.
ii. Typical crescents ..	*Vivax* or *malariae* at any stage beyond the young trophozoite.

(*b*) Difficult to identify both species :

Dominant.	Secondary.
i. *Falciparum* trophozoites	*Malariae*: full grown trophozoites or gametocytes are likely to be confused with rounded *falciparum* gametocytes.
ii. *Malariae*	*Falciparum*: rounded *falciparum* gametocytes may be mistaken for advanced quartan trophozoites or for quartan gametocytes.
iii. Segmenting *malariae* ..	Early segmenting *vivax*.

(c) Impossible to identify both species when the secondary infection is light :

	Dominant.			Secondary.
i.	*Vivax*			Young trophozoites of *falciparum* or *malariae.*
ii.	*Falciparum*			Young trophozoites of *vivax* or *malariae.*
iii.	*Malariae*			Young trophozoites of *vivax* or *falciparum.*

There are other possible combinations, but in these, as in all mixed infections, the facility with which diagnosis can be made depends on the number and distinctiveness of the forms available for examination and, not less than in all microscopic diagnosis, on the experience of the observer.

3. THE ACCURACY OF THE THICK-FILM DIAGNOSIS OF MALARIA.

The statement is sometimes made that for malarial diagnosis the examination of thick blood films is less reliable than that of thin films. The writer does not accept this view. Thick films do not lend themselves to the same accuracy as thin films for the identification of species, but they are considerably more accurate than thin films for establishing a diagnosis of malaria. Many factors—the experience of the observer, the quality of the staining, the standard of microscopy, etc.—determine the reliability of thick-film diagnosis but, granted adequate practice and careful technique, parasites may often be found in thick films where thin films have failed to reveal their presence after prolonged search. Species diagnosis from thick films, on the other hand, cannot be brought to fine limits of accuracy where infections are light.

Tables V and VI afford an indication of the diagnostic accuracy of thick film methods under two sets of conditions—(a) in cases where there are parasites enough to cause fever, and (b) where numbers are below the febrile threshold.

TABLE V.

Diagnostic Accuracy of Thick and Thin Blood Films in 500 cases of Acute Malaria.[*]

	P. falciparum.	P. vivax.	P. malariae.
Cases examined	348	111	15
Complete agreement between thick and thin films	328	103	11
Negative thin film : positive thick	16	5	3
Positive thin film : negative thick...	0	0	0
Disagreement in species diagnosis	4	3	1

[*] Examinations limited to 100 thin or 50 thick film fields.

TABLE VI.

Species Distribution in Thick Films
from 1,000 Afebrile Parasite Carriers
Indicating Proportion Unidentified.*

Positive films examined	1,000
P. falciparum	287
P. vivax	494
P. malariae	122
Mixed	15
Species unidentified ...	82

* Examinations limited to 50 thick film fields.

The films from afebrile carriers were taken at routine blood surveys. They were not checked by parallel examination of thin films but a diagnosis " species unidentified " was made whenever the species was open to reasonable doubt.

From these tables it appears that where infestation is light there is a fair expectation of about 90 per cent. accuracy in species identification from thick films, but considerably greater accuracy, approaching 100 per cent., where parasites are numerous enough to cause fever.

Mixed infections account for many of the failures to identify parasites. Table VII affords an indication of the probable error.

TABLE VII.

Diagnostic Accuracy of Thick and Thin Film Diagnosis in sixty-six Mixed Infections.

	Cases.	Thin Films.		Thick Films.	
		Both species.	One species only recognized.	Both species.	One species only recognized.
P. falciparum + P. vivax...	60	56	4	42	18
P. vivax + P. malariae ...	4	4	0	0	4
P. falciparum + P. malariae	2	2	0	1	1

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—— & ——. (1940). Ibid., 33, 507.
—— & ——. (1941). Ibid., 34, 297.
KNOWLES, R. & DAS GUPTA, B. M. (1930). Indian med. Gaz., 65, 301.
YOUNG, M. D., STUBBS, T. M. & COATNEY, G. P. (1940). Amer. J. Hyg., 31 (2), 51.

Note.—It is seldom possible to give a representative range of appearances in a single photomicrograph—hence the addition of supplementary drawings. Most of the drawings included in this series of plates were traced in the first place from photographs, though not necessarily from the photographs reproduced.

PLATE XIII.

FIG. 1.—Young trophozoites of *P. falciparum* with one leucocyte.

This is the stage of growth at which the asexual forms of *P. falciparum* are ordinarily seen in thick blood films. The parasites here are of average size ; they may be considerably larger (Plate XV and XVII, Figs. 7 and 15)* or somewhat smaller.*

Specific diagnosis at this stage is based on the small size ; the comparative uniformity ; the tendency for the cytoplasm to form definite patterns—" rings ", " commas ", " exclamation marks," etc. ; the rarity of advanced trophozoites and of schizonts ; the tendency to large numbers. Diagnosis may be assisted by association of the young trophozoites with distinctive gametocytes.

Difficulties arise mainly when numbers are small and gametocytes are absent. Solitary trophozoites cannot be distinguished from *P. malariae*, or, when large, from *P. vivax*.

FIG. 2.—Young trophozoites of *P. vivax* with one early schizont and one leucocyte.

Young unpigmented trophozoites are seldom the only forms of *P. vivax* present ; they are nearly always associated with older pigmented forms, schizonts or gametocytes, maybe with all three.

Specific diagnosis is based on the tendency to dispersion of the cytoplasm, with great variety of cytoplasmic pattern ; the tendency to larger size and smaller numbers than are usual with *P. falciparum* ; and the presence, as a rule, also of pigmented trophozoites, schizonts or gametocytes. In heavily-stained films and in films which have been kept for several days before staining the " ghost " of the enlarged host cell and the persistance of Schüffner's dots may assist diagnosis.

Difficulties arise mainly when numbers are small and other forms are absent. Solitary unpigmented trophozoites cannot be distinguished with certainty from the larger " ring " forms of *P. falciparum* or even from *P. malariae*.

FIG. 3.—Young trophozoites and two schizonts of *P. malariae*.

The unpigmented trophozoite phase of *P. malariae* is brief—about 18 hours in a cycle of 72. " Ring " forms are thus less common than pigmented trophozoites which have a growth period of 40 to 50 hours or more. Films taken at the " ring " phase usually show also a few mature schizonts and/or older pigmented trophozoites.

Specific diagnosis is based on the large size of the chromatin dot in relation to the amount of cytoplasm, the tendency to small numbers and the association, as a rule, with distinctive pigmented trophozoites or schizonts. Pigment formation in *P. malariae* is very early and a pigment haze may sometimes be detected in the larger rings.

Difficulties arise mainly when numbers are small and pigmented trophozoites and schizonts are absent. Isolated " ring " forms cannot be distinguished with certainty from *P. falciparum* and *P. malariae*.

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* The *falciparum* rings in Figs. 1, 4 and 15 were photographed at the same magnification. Comparison of their form and size is of interest. The smaller forms have a tendency to appear in the peripheral blood early in the course of infection ; the larger forms as infection advances. But there are exceptions to this rule and the possibility that there are two types of *P. falciparum* biologically and morphologically distinct is not excluded.

FIG. 1.—*P. falciparum.*

FIG. 2.—*P. vivax.*

FIG. 3.—*P. malariae.*
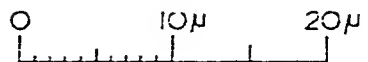
O   IOµ   2Oµ

PLATE XIII.

YOUNG TROPHOZOITES IN GIEMSA-STAINED THICK BLOOD FILMS

Note.—It is seldom possible to give a representative range of appearances in a single photomicrograph—hence the addition of supplementary drawings. Most of the drawings included in this series of plates were traced in the first place from photographs, though not necessarily from the photographs reproduced.

PLATE XIII.

FIG. 1.—Young trophozoites of *P. falciparum* with one leucocyte.

This is the stage of growth at which the asexual forms of *P. falciparum* are ordinarily seen in thick blood films. The parasites here are of average size ; they may be considerably larger (Plate XV and XVII, Figs. 7 and 15)* or somewhat smaller.*

Specific diagnosis at this stage is based on the small size ; the comparative uniformity ; the tendency for the cytoplasm to form definite patterns—" rings ", " commas ", " exclamation marks," etc. ; the rarity of advanced trophozoites and of schizonts ; the tendency to large numbers. Diagnosis may be assisted by association of the young trophozoites with distinctive gametocytes.

Difficulties arise mainly when numbers are small and gametocytes are absent. Solitary trophozoites cannot be distinguished from *P. malariae*, or, when large, from *P. vivax*.

FIG. 2.—Young trophozoites of *P. vivax* with one early schizont and one leucocyte.

Young unpigmented trophozoites are seldom the only forms of *P. vivax* present ; they are nearly always associated with older pigmented forms, schizonts or gametocytes, maybe with all three.

Specific diagnosis is based on the tendency to dispersion of the cytoplasm, with great variety of cytoplasmic pattern ; the tendency to larger size and smaller numbers than are usual with *P. falciparum* ; and the presence, as a rule, also of pigmented trophozoites, schizonts or gametocytes. In heavily-stained films and in films which have been kept for several days before staining the " ghost " of the enlarged host cell and the persistance of Schüffner's dots may assist diagnosis.

Difficulties arise mainly when numbers are small and other forms are absent. Solitary unpigmented trophozoites cannot be distinguished with certainty from the larger " ring " forms of *P. falciparum* or even from *P. malariae*.

FIG. 3.—Young trophozoites and two schizonts of *P. malariae*.

The unpigmented trophozoite phase of *P. malariae* is brief—about 18 hours in a cycle of 72. " Ring " forms are thus less common than pigmented trophozoites which have a growth period of 40 to 50 hours or more. Films taken at the " ring " phase usually show also a few mature schizonts and/or older pigmented trophozoites.

Specific diagnosis is based on the large size of the chromatin dot in relation to the amount of cytoplasm, the tendency to small numbers and the association, as a rule, with distinctive pigmented trophozoites or schizonts. Pigment formation in *P. malariae* is very early and a pigment haze may sometimes be detected in the larger rings.

Difficulties arise mainly when numbers are small and pigmented trophozoites and schizonts are absent. Isolated " ring " forms cannot be distinguished with certainty from *P. falciparum* and *P. malariae*.

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* The *falciparum* rings in Figs. 1, 4 and 15 were photographed at the same magnification. Comparison of their form and size is of interest. The smaller forms have a tendency to appear in the peripheral blood early in the course of infection ; the larger forms as infection advances. But there are exceptions to this rule and the possibility that there are two types of *P. falciparum* biologically and morphologically distinct is not excluded.

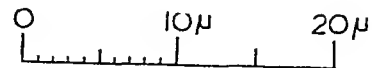FIG. 1.—*P. falciparum.*

FIG. 2.—*P. vivax.*

FIG. 3.—*P. malariae.*

O   10μ   20μ

PLATE XIII.

YOUNG TROPHOZOITES IN GIEMSA-STAINED THICK BLOOD FILMS.

PLATE XIV.

FIG. 4.—Young and advanced trophozoites of *P. falciparum* with two leucocytes.

Advanced trophozoites of *P. falciparum* with commencing formation of pigment are rarely seen except with heavy infection ; they are nearly always associated with large numbers of " ring " forms ; the start of quinine treatment sometimes seems to stimulate their appearance.

Specific diagnosis is based on the associated presence of many young trophozoites ; the compact, though maybe peripherally eroded, cytoplasm ; the irregularly circular or oval form.

The general microscopic picture renders recognition relatively easy—small rounded parasites with a haze of pigment in the cytoplasm, with undivided chromatin and with the " ring " vacuole lost or almost lost, accompanied—the most important diagnostic aid—by many young trophozoites.

FIG. 5.—Advanced trophozoites of *P. vivax* with one schizont, several young trophozoites and three leucocytes.

A tendency for the cytoplasm to disperse into delicate wisps and strands is perhaps the most distinctive feature of advanced trophozoites of *P. vivax*. The breaking up of the cytoplasm · into a cluster of fragments round the chromatin dot which is common with *P. vivax* at this stage is not seen with other species.

Specific diagnosis at this stage is based on the size ; the tendency for the cytoplasm to disperse and the great variation of cytoplasmic pattern ; the character of the pigment— —delicate yellow rodlets and granules scattered through the cytoplasm or lying free on the film background ; and the tendency to association with younger trophozoites, schizonts and/or gametocytes.

Difficulties arise when infections are very light. Isolated forms not normally dispersed may be mistaken for *P. malariae* ; if the chromatin is not visible and the pigment is not clear they may be confused with blood platelets or with the reticular debris of immature erythrocytes. With good microscopy the presence and the character of the pigment should, as a rule, obviate these sources of error.

FIG. 6.—Relatively advanced trophozoites of *P. malariae* with one leucocyte.

Pigmented trophozoites of *P. malariae* have little tendency to disperse but remain rounded and compact—like marbles in a ring. They are the commonest form of *P. malariae* seen in routine blood examination as this stage lasts for nearly two-thirds of the 72-hour schizogony cycle.

Specific diagnosis is based on the rounded or ovoid form ; the compact texture ; the early formation and the profusion of fairly coarse granules of pigment.

Difficulty in diagnosis may arise where numbers are small. Chromatin division is relatively late and large trophozoites when isolated cannot be distinguished from gametocytes. Large trophozoites may also be confused with *falciparum* gametocytes which have rounded off (Plate XVII, Fig. 15), but can be identified by the difference in the character of the pigment.

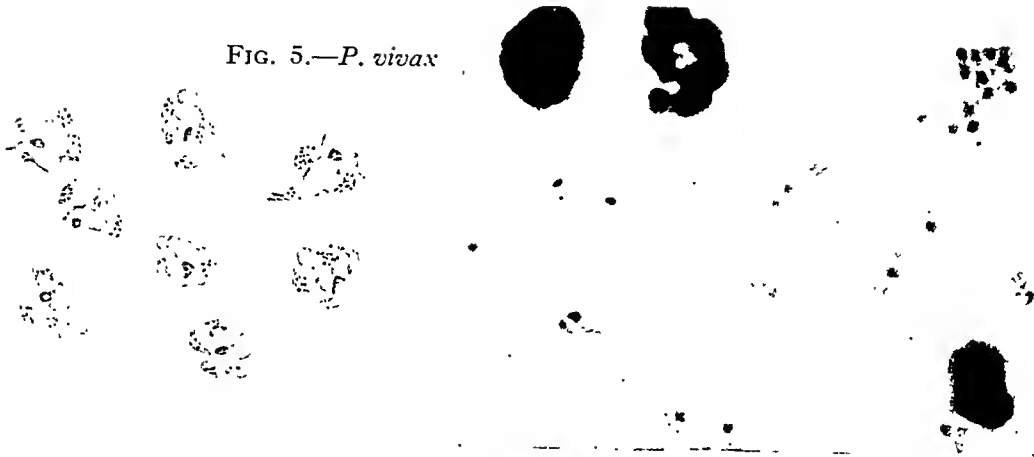FIG. 4.—*P. falciparum.*

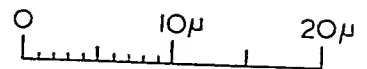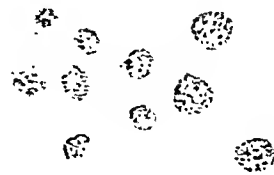FIG. 5.—*P. vivax*

FIG 6.—*P. malariae*

0　　　　　10μ　　　　　20μ

PLATE XIV

ADVANCED TROPHOZOITES WITH COMMENCING PIGMENT FORMATION IN GIEMSA-STAINED
THICK BLOOD FILMS.

PLATE XV.

Fig. 7.—Schizont of *P. falciparum* with many young trophozoites.

The schizonts of *P. falciparum* are always associated with large numbers of young trophozoites.

Specific diagnosis is based on the size ; the single dense mass of pigment ; the presence of from ten to twenty-four tiny merozoites in a close-knit cluster ; and, above all, on the association with numerous young trophozoites.

Falciparum schizonts are rarely, if ever, seen isolated in thick films from the peripheral blood and difficulties in specific diagnosis should not arise.

Fig. 8.—Schizonts of *P. vivax* with one gametocyte, one trophozoite and several leucocytes.

The schizonts of *P. vivax* are nearly always associated with other distinctive forms—trophozoites or gametocytes.

Specific diagnosis is based on the relatively large size of the schizont and of individual merozoites ; the number of merozoites—from eight to eighteen ; the small rodlets and granules of pigment, which, though concentrated, have little tendency to coalesce to a single mass ; and the association with specifically distinctive trophozoites and gametocytes.

Diagnostic difficulties may arise in very light infections. Young schizonts with less than ten chromatin segments may be confused when isolated with *P. malariae* : assistance in differentiation is afforded by the greater tendency for the merozoites of *P. malariae* to disperse and by the differences in the character of the pigment.

Fig. 9.—Schizonts of *P. malariae* with one leucocyte.

Mature schizonts of *P. malariae* fairly regularly have eight cleanly-separated merozoites and a single collection of coarse pigment granules ; they are usually accompanied by a few young trophozoites. The chromatin of the merozoites may be clothed with cytoplasm but often, as in two of the drawings, is bare and isolated.

Specific diagnosis is based on the small size ; the presence of about eight merozoites ; the tendency to dispersion of the merozoites ;* the appearance of the cluster of coarse pigment granules ; the association with other distinctive forms somewhat less or more advanced in development.

Diagnostic difficulty arises only when infections are light. Isolated schizonts are easily confused with immature schizonts of *P. vivax* and are differentiated mainly by the differences in the pigment. The schizonts of *P. falciparum* are seen only with an intensity of infection that *P. malariae* rarely, if ever, attains.

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* The tendency for the merozoites of *P. malariae* to scatter more widely than those of *P. falci-parum* or *P. vivax* is indicated in the drawings but not in photographs.

FIG. 7.—*P. falciparum.*

FIG. 8.—*P. vivax.*

FIG. 9.—*P. malariae.*
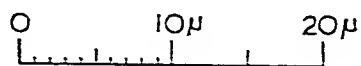
PLATE XV.

SCHIZONTS IN GIEMSA-STAINED THICK BLOOD FILMS.

PLATE XVII.

FIG. 13.—Immature gametocytes of *P. falciparum* with many young trophozoites.

Immature *falciparum* gametocytes in thick blood films are often long and slender with pointed ends and scattered pigment. They are likely to be found only in the early days of attacks and to be associated with many young trophozoites.

Species diagnosis is based on the characteristic shape and the presence of numerous young trophozoites.

Diagnosis difficulty is not likely to arise if the distinctive thick-film appearance of these young forms is recognised.

FIG. 14.—Three ovoid mature *falciparum* gametocytes with young trophozoites and one leucocyte.

FIG. 15.—Two rounded *falciparum* gametocytes with young trophozoites.

Mature *falciparum* gametocytes often pass through the first stage of maturation during the slow drying of thick blood films ; they contract, become ovoid and finally round.

Specific diagnosis is based on the character of the pigment—coarse rodlets, discrete but with a tendency to concentrate near the centre ; and on the fact that characteristic sausage-shaped gametocytes, or young trophozoites are often also present.

Diagnostic difficulty arises only with light infection. Isolated ovoid or rounded gametocytes of *P. falciparum* closely resemble large trophozoites and gametocytes of *P. malariae* and are often confused with them. Differentiation may depend solely on the differences in the pigment and is not always easy.
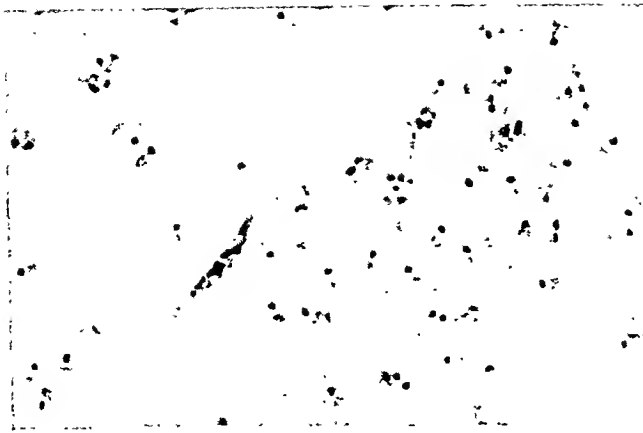
FIG. 13.
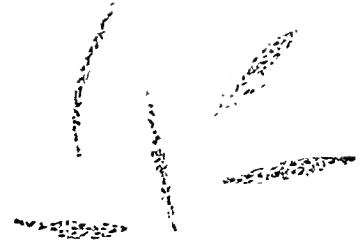
FIG. 14.

FIG. 15.
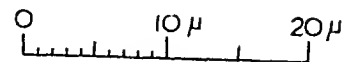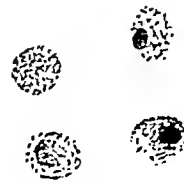
PLATE XVII.

VARIOUS FORMS OF *P. falciparum* GAMETOCYTES IN GIEMSA-STAINED THICK BLOOD FILMS

ARTIFICIAL INFECTION AND IMMUNIZATION OF MAN WITH CULTURES OF *LEISHMANIA TROPICA*.

BY

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INTRODUCTION.

MANSON (1914) stated that it was a custom among the Jews of Baghdad to inoculate their children on the covered parts of their body with material taken from a Baghdad boil, with the object of immunizing them and preventing the development of unsightly scars on the face or hands.

WENYON (1911) was assured by a doctor in Mosul that he had practised this method of inoculation thirty-six times with satisfactory results, the boil appearing in about two months.

We have been told by doctors long established in Baghdad that the procedure is still carried out and that they have seen cases. Our own efforts to find persons who have been thus inoculated have proved unsuccessful, and we must conclude that, if still practised, it is uncommon.

The naturally occurring Baghdad boil is generally single. Rarely are multiple infections found, and still more rarely, reinfection. For these reasons it would appear that artificial inoculation should, in the majority of cases, produce an immunity and prevent the subsequent development of the natural disease.

The use for this purpose of material taken from a Baghdad boil is, however, open to criticism. Pyogenic infections, malaria and syphilis may be so transferred and the dosage of the inoculated material containing the organisms is unknown. Moreover, material rich in leishmania is not always available.

It would be more satisfactory if the Baghdad boil could be produced with culture material. In 1910 NICOLLE and MANCEAU showed that this could be done. By scarification they obtained doubtful results, but by intradermal injection they produced a typical lesion after an incubation period of 6 months.

The largest experiment so far carried out on these lines was that of LAWREW and DUBOWSKOJ (1937). They inoculated 487 persons with cultures of *Leishmania tropica*, but were able to follow up only 227. Of these 164 developed an oriental sore, giving 73 per cent. positive results. These workers used for most of their inoculations 6 to 10 day old cultures, but in a few cases used 20 to 30 day old cultures ; 85 per cent. of the positive results were obtained with cultures in their first or second generation. They considered that later generations might lose their infectivity. The incubation periods observed by them were :—

> 2 to 3 months in forty-five cases,
> 4 to 6 months in eighty-four cases,
> 7 to 9 months in thirty cases,
> 10 to 12 months in nine cases.

Recently BERBERIAN (1939) has reported the inoculation with living cultures of *L. tropica* of thirty-four volunteers who had no history of past infection of Baghdad boil. Five of these received more than one injection. The percentage of positive results was eighty-eight and the incubation period observed varied from 2 weeks to 5 months for the intracutaneous, and 3 to 6 months for the subcutaneous, inoculation.

The object of the present paper is to report on the methods we have used in carrying out inoculations with cultures of *L. tropica*, to record the results we have obtained and to attempt to assess the value of the procedure in producing immunity.

Material and Methods.

Locally isolated strains of *L. tropica* grown on the medium previously described (SENEKJI, 1939) were employed. Before the cultures were used smears were made from them and examined for bacterial contamination. If they proved free from bacteria, they were emulsified in sterile saline. A leishmania count was carried out either in a blood-counting chamber or by comparison with Brown's opacity tubes (SENEKJI, 1939). The suspension was then diluted with saline to contain 20 million leptomonads per c.c.

In 1938 we used a polyvalent suspension of, on an average, ten strains which had been grown for 3 to 4 weeks. In cultures of this age post-flagellated forms were abundant. In 1939 and 1940 we used a single strain (Nahid strain isolated in 1938), which had been grown for 4 to 6 days. Such young cultures contained only actively motile leptomonads.

Inoculation was carried out on the anterior surface of the left thigh which

was first thoroughly cleansed with alcohol and the alcohol allowed to evaporate in order that it might not injure the leptomonads. The leishmania suspension was then aspirated with a sterile and cold syringe and through a fine intradermal needle 0·15 c.c., containing 3 million leptomonads, was injected into the skin. The same dose was given to all regardless of age. In order to be able to observe the development of the artificially produced boil, measurements were taken and recorded of the distance of the point of inoculation from the superior edge of the patella.

Results.

Since 1938 we have inoculated 227 persons, none of whom had previously a Baghdad boil. In this number are included Iraqis, Syrians, Palestinians, Turks, Iranians, Egyptians, Rumanians, Hungarians, Armenians, Germans, Americans and Britons. Forty-one persons were inoculated in 1938 with polyvalent strains of 3 to 4 weeks old cultures, while in 1939 and 1940, 186 persons

TABLE I.

Incubation Period.	Adults.	Children.	Total.
Less than 2 weeks ...	17	—	17
2 weeks	25	6	31
3 ,, ...	70	1	71
4 ,, ...	33	4	37
5 ,, ...	—	1	1
6 ,, ...	3	5	8
7 ,, ...	—	3	3
8 ,, ...	2	28	30
Total	150	48	198
1st inoculation negative 2nd inoculation positive	2	—	2
Grand total ...	152	48	200

were inoculated with 4 to 5 day old cultures of the Nahid strain. We were able to follow up 200 cases. Of this number 48 were children below 5 years of age and 152 were adults.

The incubation period is shown in Table I, from which it will be seen that the average incubation period in adults was from 2 to 4 weeks and in children about 2 months.

With a single injection the percentage of positive takes was 99 while with two injections it was 100.

The typical course of the artificial boil appeared to be that after about three weeks a macule appeared which later became papular and had a purple colour. There was infiltration of the base and slight scaliness of the surface ; in 6 months it became as large as a coffee bean. About 50 per cent. of the boils ulcerated in 6 to 8 months, and the ulcer healed in 9 to 12 months. It is our belief that ulceration is in most cases due to trauma, and that if trauma is avoided the boils do not ulcerate.

The course taken by the artificially produced boils observed by us corresponds to that described by BERBERIAN (1939).

Three individuals, one child and two adults, developed a natural boil shortly after the appearance of the artificial boil.

In order to attempt to determine the immunizing value of artificial inoculation, we reinoculated two individuals who had artificial boils in the unhealed stage, and one individual who had both artificial and natural boils in the unhealed stage and five individuals who had natural boils in the unhealed stage. All eight developed an artificial boil in response to inoculation.

In contrast to this, four individuals who had healed artificial boils and fifteen adults who had had natural Baghdad boils in childhood, did not develop an artificial boil on inoculation with cultures. Instead, an acute inflammatory reaction developed immediately, accompanied by a rise of temperature (38·5° C.), and in some cases by slight feeling of chilliness and by enlargement and tenderness of the regional glands. These reactions subsided in 48 hours. Again our results correspond to those of BERBERIAN (1939).

DISCUSSION.

It was our object to produce an artificial Baghdad boil in all the previously uninfected persons we inoculated. In this we succeeded, showing that there is no absolute natural immunity, but our success we attribute to the fact that we used massive doses of *Leishmania* cultures and gave it intradermally. This being so, we feel that we are not in a position to make any contribution to the controversy concerning what generations, age of cultures or strains are most infective. (Row, 1939 ; ADLER and THEODOR, 1939 ; BERBERIAN, 1939). All that we can say is that with the method used by us it seemed immaterial whether recent or old generations, young or old cultures, one or more strains were used. A solution of the question awaits the accurate estimation of the minimum infective dose of different generations of cultures and strains. We were at first somewhat disappointed to find that three persons developed a natural boil after the appearance of the artificial boil. On inquiry we found that these were persons in whom the artificial boil was as yet unhealed.

We therefore endeavoured to ascertain when immunity was produced after natural and artificial boils. From the experiments described, in which by reinoculation of persons with unhealed natural or artificial boils we could

produce another boil, while on inoculation of persons with healed boils we produced what appeared to be an allergic reaction, we would suggest that immunity is slow in developing and is not complete until the boil has healed.

Our experience leads us to recommend further trial of immunization by means of live cultures in places where cutaneous leishmaniasis is prevalent. We would, however, give a word of warning. The site chosen by us was frequently subject to trauma, particularly in athletic people. As a result, in some cases large ulcerated boils developed. We would recommend that in future work a less exposed site should be selected.

Summary and Conclusions.

1. A method is described of producing Baghdad boil by the inoculation of culture material.

2. 227 persons were inoculated but only 200 followed up. In all of these a boil was successfully produced, although in two cases a second injection was needed. The average incubation period was in the adult 2 to 4 weeks, and in children about 2 months.

3. Three of those inoculated subsequently developed a natural boil, but in none of them had the artificial boil healed.

4. Re-inoculation of persons who had unhealed boils resulted in the production of another boil.

5. Inoculation of persons who had healed natural or artificial boils resulted in a reaction of allergic type.

6. We suggest there is no absolute natural immunity to Baghdad boil. Immunity follows infection and takes a considerable time to develop, not being complete until the boil has healed.

7. In places where cutaneous leishmaniasis is prevalent immunization with live cultures of *Leishmania tropica* is worth a trial.

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AGRANULOCYTOSIS IN KALA-AZAR AND USE OF ADRENALIN.

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Kala-azar is not commonly seen in England, although it is possible that a certain number of cases may remain undiagnosed. The disease is, however, not particularly rare among seamen, and the majority of patients suffering from it are Indians. It is, therefore, natural that it should be the Indian variety of the disease from the eastern side of that country, which is mostly represented. Since September, 1939, five cases of this disease have come under our observation, but it is proposed here to give a detailed report of one case only in which the complication of agranulocytosis was present. All of the cases which have come under our care, have shown some generalized lymphatic glandular enlargement, although this manifestation is generally supposed to be uncommon in adult Indian leishmaniasis. The glandular enlargement is not very great, and the

glands are most commonly found in the inguinal and cervical regions. The epitrochlear glands are not usually found to be enlarged. Although the disease presents certain clinical characteristics which may render the diagnosis probable, it is, nevertheless, essential that no case should be accepted as proven, until the characteristic leishmania have been recovered.

CONFIRMATION OF DIAGNOSIS.

Leishmania are present in the bone-marrow, in the spleen, in the liver and in the lymphatic glands, and according to ZIA and FORKNER (1934), even in the nasal secretion. As a general statement it may be said that the parasites are arrested by the cells of the reticulo-endothelial system. Various methods have been used in our series for the recovery of leishmania, but splenic puncture was not utilized, although this is probably the method which has been most frequently used in the past. Hepatic puncture proved simple and reliable. Bone-marrow puncture presents certain minor technical difficulties, but when these have been overcome it is safe and reliable, and remarkably well tolerated by the patient. Sternal puncture was the method which we used. Puncture of the lymphatic glands has only recently achieved the prominence which it deserves. COCHRAN (1912), described the recovery of leishmania from the smears made from excised lymph nodes. GIRAUD, MONTUS, SARDOU and GAUBERT (1936), reported their successful results in gland puncture, and GIRAUD, BOUDOURESQUES, BLANC and BERGIER (1937), described two further successful cases. D'OELSNITZ (1934), also lays emphasis on the value of this diagnostic method, and in particular draws attention to the simplicity of puncture of the epitrochlear glands. It is noticeable that between the work of COCHRAN (loc. cit.) and the work of GIRAUD et al. (loc. cit.), glandular enlargement does not appear to have aroused any interest. KIRK and MOHAMMED HAMAD SATI (1940), reported their results in the diagnosis of kala-azar in the Egyptian Sudan. They were uniformly successful in recovering leishmania by means of gland puncture. They recommended that the lower group of superficial inguinal glands disposed vertically along the terminal part of the long saphenous vein should be selected, since these glands can be easily palpated and immobilised by a firm grip between the thumb and fingers. The skin is sterilized and the gland is pulled up from the underlying tissues and steadied. A dry sterile No. 16 hypodermic needle is pushed through the skin and into the gland. KIRK and SATI attached no syringe to the needle, as they found that the juice runs up the needle very easily of its own accord, and that it is usually not difficult to feel when the needle has entered the gland. They recommend that the needle should be held in position for a second or two and then quickly withdrawn. A small syringe is then attached so that the gland juice in the needle can be blown out on to a slide and stained. We have found it somewhat easier to attach the needle to a sterile dry syringe with a loosely fitting plunger already slightly withdrawn, as this method gives better control

of the needle during the act of puncture. In our small series of cases, we are able to confirm the uniform success which KIRK and SATI reported, and we regard the method of lymphatic gland puncture as being an entirely satisfactory procedure for the recovery of the leishmania. We found that leishmania could be recovered from glands which, although a little enlarged by European standards, were not beyond the limits which we regard as normal among Indian seamen.

INCIDENCE OF AGRANULOCYTOSIS.

ZIA and FORKNER (1932), note that a chronic leucopenia with leucocytes varying from 1,500 to 5,000 cells per c.mm., and with granulocytes from 20 per cent. to 60 per cent. is the rule in kala-azar. In their original paper, these authors express the belief that acute agranulocytosis had not, up to that time, been reported as a complication of kala-azar. From time to time, cancrum oris and noma had been described as terminal complications of kala-azar. It does not seem unlikely that these terminal complications were, in fact, unrecognized agranulocytosis. They observed four cases of agranulocytosis among twenty-six cases of kala-azar which came under their care during a period of 8 months. The first of these four patients developed agranulocytosis towards the end of a course of neostibosan, was treated with pentnucleotide and made a complete recovery. The second patient also developed acute agranulocytosis at the end of a course of treatment with neostibosan, and this patient was treated by means of blood transfusion and also recovered completely. Their third case was that of an infant who had not received any antimony treatment, but in whom the kala-azar was complicated by diphtheria. Pentnucleotide proved to be of no benefit, but three small transfusions produced so great an improvement that it was possible to proceed with a course of neostibosan, by means of which the kala-azar was cured. The fourth case which these authors discuss was already moribund on admission, and showed extreme leucopenia with complete absence of granulocytes. At postmortem tremendous proliferation of macrophages with ingested leishmania was found. The patient was a boy of 9½ years, and STRONG (1920), drew attention to the fact that the reduction of neutrophiles in kala-azar was more marked in children than in adults. In the light of the postmortem findings in this case, the suggestion made by HU and CASH (1927) that the anaemia in kala-azar is myelophthisic, in the sense that there is a crowding out of blood forming tissues by the tremendous increase of macrophages, would appear to have some confirmation. ZIA and FORKNER speculate that the extreme leucopenia and the total absence of granulocytes in certain of their cases may be, at least in part, the result of this process. TSO (1931), reported a case which presented a chronic granulocytopenia. ZIA and FORKNER (1934), recorded a further four cases of acute agranulocytosis, making a total of eight cases. Analysing these cases, it would appear that recovery occurred in five instances, death in two, while one was discharged from hospital contrary to medical

advice, and her fate is not known. Three of the eight cases were treated with neostibosan and three with ureastibamin, before the development of acute agranulocytosis. Two of the cases only, received no antimony.

TREATMENT OF AGRANULOCYTOSIS.

TAUSSIG and SCHNOEBELEN (1931), show that untreated cases of agranulocytic angina have a mortality of 75 per cent., while in patients treated by means of blood transfusion, the mortality fell to 64 per cent. Many investigators, particularly FRIEDEMANN and ELKELES (1930), treated the condition with small doses of X-ray, and the collected series of TAUSSIG and SCHNOEBELEN showed a mortality of 53 per cent. when this treatment was used. The use of various sera, liver extract, foreign proteins, etc., have all been reported, but the small number of cases discussed makes it impossible to draw any conclusion from the findings. REZNIKOFF (1933), reported certain derivatives of nucleic acid as having the power to raise the peripheral count, and by means of this treatment the mortality was reduced to 27 per cent. of thirty-five cases. JACKSON, PARKER and TAYLOR (1932), used pentnucleotide and experienced a mortality of 30 per cent. in fifty-four patients. The following points in the treatment of agranulocytosis may be regarded as useful additions to the administration of pentnucleotide :—

1. Strict respiratory isolation from the onset.
2. If the attack is associated with treatment by means of drugs (antimony, arsphenamide), such medication should be stopped.
3. Diet should be liquid, or at any rate very soft.
4. Liberal amounts of fluid should be taken.
5. Mouth washes of hydrogen peroxide and of saturated solution of sodium perborate are recommended.
6. Mucous membranes should be painted frequently with 1 per cent. aqueous solution of gentian violet.

Of the cases recorded by ZIA and FORKNER, three were treated with pentnucleotide only, two with a combination of pentnucleotide and blood transfusion, and two cases received no specific treatment for the agranulocytosis. In view of the small number of cases of agranulocytosis in kala-azar so far recorded, the following case from our own small series is reported in some detail.

CASE.

F.R.   Indian Seaman.   Aged 30 years.   Admitted 2.4.40.

On account of language difficulty, history was inadequate. Cough for 2 to 3 months and fever for 1 month.

Physical Signs.

Patient looked very ill. Temperature 104° F. on morning following admission. Pulse 114. Respiration 28. Temperature showed typical double rise and fall in 24 hours. Spleen was enlarged 3 cm. below the costal margin. Cervical and inguinal lymphatic glands were moderately enlarged.

4.4.40.—Blood count showed slight granulocytopenia (see table). No nucleated forms were seen. No malarial parasites or spirochaetes were seen.

5.4.40.—Formol-gel test positive. Ova of *Ankylostoma duodenale* and *Trichuris trichiura* were present in the stool. Leishmania were present in material from glandular and from sternal puncture. Urine showed a trace of albumin.

9.4.40.—Daily injections of 0·2 gramme neostibosan were started, and by 21.4.40, a total of 2·6 grammes had been administered, all but one of the injections being intravenous. The temperature, pulse and respirations fell progressively towards the end of the course of treatment.

21.4.40.—With temperature still normal, the patient was found to be lethargic, to have anorexia, and superficial ulcers made their appearance on the gums.

23.4.40.—Temperature rose sharply to reach 103° F. by 10 p.m. Blood count showed severe granulocytopenia. (See table.)

A diagnosis of agranulocytosis was made on the 23.4.40, as a result of the blood count. On the following day, 24.4.40, injections of pentnucleotide were

TABLE OF BLOOD COUNTS.

Date.	Time.	Red Blood Corpuscles. Per c.mm.	Haemoglobin (Haldane). Per cent.	Leucocytes. Per c.mm.	Polymorphs. Per cent.	Lymphocytes. Per cent.
4.4.40	—	3,400,000 Anisocytosis	48	2,000	40	57
23.4.40	—	3,110,000	46	1,800	3	97
25.4.40	—	3,050,000	55	1,650	2	—
26.4.40	—	3,610,000	—	1,400	1	Total Polymorphs. Per c.mm. 186
28.4.40	10.0 a.m.			1,860	1	
	After adrenalin min. xv 10.20			5,125	49	2,571
	10.30			6,725	54	3,631
	10.40			4,125	52	2,145
	10.50			3,215	53	1,703
	11.0			2,875	58	1,655
	11.10			1,475	35	516
30.4.40	10.15 a.m.			3,775	66	—
	10.35 after adrenalin min. v			4,580	67	—
1.5.40	10.30 a.m.			4,300	71	—
	10.50 after adrenalin min. v			4,475	—	—
2.5.40	10.0 a.m.			4,400	61	—
	10.20 after adrenalin min. v			7,850	—	—
17.5.40	—	3,450,000		8,100	64	—

started, and with the exception of two intravenous injections of nucleinic acid given during the morning of the 25.4.40, all the injections consisted of 10 c.c. of pentide given intramuscularly. On the evening of the 25.4.40, a blood transfusion of 100 c.c. of stored blood was given, but the patient was very restless and the transfusion was therefore abandoned. On the following day, it was possible to give 1 litre of fresh blood by slow continuous drip transfusion. On the 27.4.40, that is after 4 days, the patient began to show some slight clinical improvement, although the blood picture was still unsatisfactory on the following day. On the 28.4.40, adrenalin, 15 minims, was injected subcutaneously. It will be seen from the table of blood pictures, that before the injections of adrenalin the blood picture showed 1,860 white blood cells with only 1 per cent. of polymorphs. The patient's clinical condition gave rise to grave anxiety. The increase in white cell count is shown in tabular form, and it will be noticed that the great increase in leucocytes was accompanied by an almost exactly parallel increase in the total number of polymorphonuclear cells. One hour after the injection, the leucocyte count had fallen back to its initial figure, but it will be noted that the increase, both in the relative and the absolute polymorphonuclear values, was still to some extent maintained. In view of this response, adrenalin was given frequently during the following week in subcutaneous doses of 5 minims, and the response on 3 typical days is shown in the table. It is interesting to note that after recovery from the agranulocytosis, no further treatment was found to be necessary for the kala-azar, although the ordinary case requires at least two courses of neostibosan. It seems almost as if the acute agranulocytosis had in some way exerted a curative action upon the kala-azar.

It appears, therefore, that adrenalin produced a complete cure of agranulocytosis in a patient who had already had the condition for 5 days, and who had responded neither to pentnucleotide nor to blood transfusion. Opportunities for testing the value of adrenalin in agranulocytosis are necessarily few, but in this case, at least, it appears to have produced a specific result after the accepted remedies had failed.

It was thought, in view of the results obtained, that 5 minims of adrenalin was perhaps not sufficient dosage, and investigations recently carried out have shown that a brisk and consistent leucocytosis can be produced by adrenalin, 15 minims.

CASTRÉN (1916) and HATIGAN (1917), were the first authors to draw attention to the leucocytosis which follows an injection of adrenalin. COWIE (1919) reviewed the subject at some length, and his reported work shows that out of a total of thirteen cases injected with adrenalin, one case only failed to produce a leucocytosis. BENDA (1930) summarized the then known facts about the use of adrenalin in haemorrhagic and anaemic states. The observations, which are pertinent to this paper, are firstly based upon a case of haemorrhagic purpura, published by E. SERGENT, DURAND, GRELLETY-BOSVIEL and BENDA (1927), in which subcutaneous injections of adrenalin produced startling improvement,

but BENDA also quoted WEIL and ISCH-WALL (1929), who published a case where adrenalin was used in a case of splenomegaly, and produced a fatal haematemesis. The increase in the number of red blood corpuscles which followed the injection of adrenalin was, at that time, thought to be a constant phenomenon, and certain animal experiments appeared to indicate that this increase was mainly, if not entirely, due to the contraction of the spleen and the consequent expulsion of red blood corpuscles into the circulation. It is of great interest to note that PAGNIEZ, COSTE and ESCALIER (1925), report that although the increase of red blood cell count is tremendous and almost instantaneous, there is no parallel increase in the white blood cell count. Various French authors published conflicting results from experiments in which the red blood cell count was taken before and after the injection of adrenalin. PAGNIEZ, COSTE and ESCALIER report an increase in an animal of from six to eight million red blood cells in a period of only 1 minute, but by ordinary methods of blood counting this seems to be a technical impossibility.

BENDA himself, working with CLAUDE TESTU, found that the increase in red blood cell count was extremely variable, and he quoted four cases taken at random from his series, in two of which a small increase in red blood cells took place, while in the other two a small decrease was recorded. He draws attention to the fact that both the increase and the decrease were within the limits of experimental error. JONESCU, working with us on a similar line of experimentation, found in five successive patients suffering from malaria, that 15 minims of adrenalin given subcutaneously, produced in each case a considerable rise in the total white cell count, but no significant alteration in the differential count. These results have not yet been published. GIBSON (1926), reports the result of treatment by injection of adrenalin in a girl suffering from what was described as aplastic pernicious anaemia. In trying to explain his very satisfactory results in this case, GIBSON thought that perhaps a direct stimulation of the bone-marrow had taken place.

No further work appears to have been done on this very interesting aspect of the problem.

SUMMARY.

1. The methods of diagnosis of kala-azar are reviewed. Emphasis is laid upon the simplicity and reliability of glandular puncture.

2. The incidence of agranulocytosis in kala-azar is very small, and the literature on the subject is reviewed.

3. The treatment of agranulocytosis is discussed, with special reference to the value of adrenalin.

4. A case of kala-azar occurring in this country is described in detail, together with the treatment of the resulting agranulocytosis.

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THE INCIDENCE AND TREATMENT OF YAWS IN THE WESTERN SOLOMON ISLANDS.

BY

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INTRODUCTORY

This investigation was undertaken for the purpose of estimating the incidence of yaws in the population at large of the Western Solomons, of appraising the efficiency of the methods of treatment at present in use, and of exploring the possibility of improvements in treatment that would be practicable under the conditions existing in this region.   It is fully realized that a number of the findings are probably relevant only to the area under review, and would not be applicable to other places where conditions of life and different transport facilities obtain : the latter factor in particular is of primary importance in limiting the efficiency of treatment which can be obtained at a reasonable expenditure of time and money.

The work has been carried out over a period of 2 years at the Helena Goldie Hospital, Bilua, Vella Lavella, by the writer himself, with the aid of two qualified

nursing sisters. On the latter falls the task of organising and supervising the injection clinics, and also of attending to the keeping of the records on the rather frequent occasions of the writer's absence from hospital.

GENERAL DESCRIPTION OF THE CLINICAL MATERIAL AND METHODS.

The figures from which the various tables are compiled have been obtained by a careful analysis of the record cards of all patients seen personally at the central hospital, Bilua, during the period from June 1st, 1938, to March 31st, 1940.

Most of the patients at this clinic are drawn from the two islands of Vella Lavella and Ranono in the Western Solomon Islands. These are two small islands, about 25 and 15 miles long respectively, lying close together at about lat. 8° S. and long. 157° E. The entire population, of about 2,600 in all, lives in small villages—of fifty to a hundred people—at short intervals around the indented coast line. Bush villages in the inland mountains no longer exist. The two islands are typical of all other islands in the group.

About a quarter of the patients seen at the clinic come from other islands of the group, at varying distances up to a hundred miles and more from hospital. Except for natives living in a few villages on Vella Lavella itself, within a few miles of hospital, all travel is by native canoe, through stretches of sea that are often dangerous, and sometimes quite impossible of passage. The canoes are dugout canoes of varying sizes, carrying from three to twelve people, occasionally more. Patients from the more distant islands take advantage of launches, either those of the Mission or of Chinese traders, to make the trip, but very occasionally a large canoe may come as far as from the northern coast of Choiseul, at a distance of 120 miles.

Patients coming from distances of more than a few miles usually arrange to stay at Bilua for periods of 2 to 4 weeks while receiving treatment; only rarely will they stay longer, owing to the difficulty of arranging food supplies for a longer period. For the shorter distances, including the villages on the near side of Ranono, canoes will sometimes make weekly trips for injections; but here again enthusiasm wanes after 2 or 3 weeks, or weather prevents the making of the trip, and treatment is similarly interrupted or curtailed. This very important factor is discussed again later.

The patients are nearly all full-blooded Western Solomon Islanders. They live in leaf houses, which at their best are clean, airy, and raised off the ground on piles; but at their worst are small, dark, and without flooring. Infants and small children are usually unclothed in the villages; older boys wear a " tivi " or loincloth, and the girls a dress with usually knickers or a loincloth underneath. Adult men usually wear only a loincloth, but the women tend to overdress, wearing several undergarments often dirty, beneath a dress which may itself be kept fairly clean. Teachers in charge of villages are all supplied

with bandages and medicines, and are expected to see that all ulcers are kept covered ; frequently, however, they fail in this respect, especially in cases of extensive ulceration. Conditions for 'fly transmission of infection are therefore favourable.

Malaria is endemic and, though only occasionally severe, is an important cause of chronic ill-health and lowered resistance to disease. The diet in the villages is mainly vegetable—taro, sweet potato, banana, pawpaw, native spinach and the like, with a variable amount of fish. There is an increasing tendency to use rice. Both proteins and fats would appear to be deficient in many cases, and the writer is of opinion that this is an important factor in lowering resistance to yaws infection and in hindering the efficacy of injected drugs. Scabies is rife and scabies lesions must frequently provide the port of infection for the yaws organism, as well as causing secondary pyogenic infections in existing yaws ulcers.

<div align="center">TREATMENT.</div>

Daily dressings with eusol, acriflavine, or brilliant green are given at the Outpatients' Clinic, and other local measures instituted when necessary. Adjuvant treatment with quinine, iron, cod-liver oil, calcium etc., is given when the clinical condition indicates. Potassium iodide is used only for patients with frank bony lesions, or occasionally, and more or less empirically, for recalcitrant ulceration.

All babies and children are treated with sobita—sodium bismuthyl tartrate —in watery solution, the usual dosage being as follows :—

Children up to 9 years	0·1 c.c. per year of age, plus 1.
Children 10 to 13 years	1·0 c.c.
Older children ..	1·2 c.c.
Adult women	1·4 to 1·6 c.c.
Adult men	1·6 to 1·8 c.c.

Injections are given usually at weekly intervals, occasionally at 5-day intervals. The injection is deep into the gluteal muscles, and is given by trained native orderlies. In a total of over 4,000 injections given at the hospital during the period under review, no case of abscess due to the injection has occurred. Large numbers of injections are also given by sisters stationed at branch hospitals on the islands of Roviana and Choiseul, and by trained native dressers at several other centres. Only one instance of abscess developing at the site of injection has come to the author's knowledge.

Adults were treated with novarsenobillon (N.A.B.) during the earlier part of the period under review, but latterly, for the reasons set out below, this drug has been increasingly, and now almost completely, replaced by sobita. Some complaints of sore buttocks used to be heard in the early days of sobita treatment (when N.A.B. was also available), but have ceased of late. Figures for N.A.B. are therefore not very large, but an attempt has been made below to evaluate the relative efficacy of the two drugs.

Whether sobita or N.A.B. is used, a minimum of three consecutive injections is always aimed at ; latterly we have tried to give four, even when the ulcer is healed after only three, in the hope of reducing the relapse rate. The efficacy of this measure is discussed below. In refractory cases as many as eight injections may be given consecutively. In all the tables set out for analysis below, the following arbitrary designations of treatment have been used :—

Three or more consecutive injections   ..   T1
Two consecutive injections only T2
One only, or injections at wider intervals   ..   T3

Treatments " T2 " and " T3 " have been grouped together in most of the analyses.

Toxic Effects. No toxic effects have been observed with N.A.B. Very occasionally a mild stomatitis has resulted from sobita, usually in adults with untreated pyorrhoea ; it has always yielded to simple measures, and practically never interferes with treatment. No cumulative effects have been observed in patients receiving up to eight consecutive injections at weekly intervals, or larger numbers over a longer period.

Records.

These are kept on cards, a card to each patient, indexed by name and village. At the first attendance notes are made of the nature and site of the lesion, the dose of the drug, treatment instituted, and any other relevant findings ; on subsequent attendances the date is noted of final healing. The exigencies of a crowded out-patient clinic, conducted in an inadequate leaf building, the rather frequent changes in the person keeping the records, and also the practice of treating many of the larger lesions with occlusive elastoplast dressings, lead to many imperfections in the records. On this account a large number of cases had to be discarded, except for the purpose of computing the incidence of the disease, in which instance details were not required ; those included in the final analysis are those only for whom adequate records have been kept.

Follow up.

This is a matter of the greatest importance, but of the utmost difficulty under island conditions. The follow-up records tend to be loaded by relapsing cases, since a number of patients not relapsing never return to hospital, and an inquiry extending through all villages could be fruitful only if each patient were personally examined—an undertaking demanding more time and money than is available. However, a number of patients do return for other conditions and so have their condition recorded on their card, and others come under personal notice, and can be included in the returns. For the purposes of this survey, patients not actually seen at hospital and those not observed for at least nine months from the initial attendance, are recorded as " not

traced." If, however, relapse is known to have occurred within 9 months, the case is naturally included in the " Relapse " figures. It was hoped at the beginning of the investigation that it might be assumed that, on Vella Lavella at least, the non-return of a patient might be taken to mean absence of relapse. Further investigation of the figures, however, and visits paid to the villages, provide ample evidence that this cannot be relied on. Every patient, therefore, whose subsequent history is not actually recorded on his card, is placed in the " Not Traced " group.

The following questions are dealt with in order, in the sections that follow :

1. The incidence of the disease in the population at large.

2. The efficacy of sobita as an agent in the treatment of secondary and early tertiary yaws in children, both as regards the immediate result and the remote prognosis.

3. The relative efficacy of the two drugs sobita and N.A.B. in the treatment of late tertiary yaws in adolescents and adults.

4. What is the usual time of relapse in treated cases, and what modifications of the standard treatment may be of value in reducing the relapse rate.

INCIDENCE.

It was hoped to provide an answer in this section to two questions, *viz.* : What proportion of the population is suffering from yaws at any one time ? And secondly, what is the common age for contracting the infection ? It proved impossible, however, to collect sufficiently accurate data for a reply to the second question.

The figures used for this computation were those applying to the islands of Vella Lavella and Ranono only, since for these two islands the hospital is the only centre at which injections are given. Other islands have their own white nurse or native dresser giving injections, and those treated at the hospital are those only who come to Bilua for other purposes, or refractory cases who have been referred to Bilua for special treatment. The numbers from these islands do not therefore reflect the actual number infected. On Vella Lavella and Ranono, on the other hand, it may be assumed that the great majority, though certainly not all, of people affected do come for treatment. They have great faith in the efficacy of injection treatment, and only in exceptional cases do they fail to come eventually—though they may suffer for months or years before so doing.

The figures represent the total number of patients belonging to the Methodist Mission, personally examined by the writer at the central hospital, Bilua, and diagnosed as suffering from active yaws infection, from June 1st, 1938, to March 31st, 1940, related for the purposes of calculation to the total Methodist population of the two islands at September 31st, 1939. A small minority of the population on both islands is not attached to the Methodist

Mission, most of them being Seventh Day Adventists ; many of these receive treatment at the Methodist hospital, but in this calculation they have been omitted from both numerator and denominator, as the proportion receiving treatment at other centres is not known. Their numbers are not great enough, however, to affect the result to a significant extent, nor is there any reason to believe that the incidence of the disease varies significantly in the two groups of the population.

The figures are as follows :—

<div style="text-align:center">

Number of cases of active yaws .. 1,141

Total Methodist population .. 2,184

</div>

Thus the percentage of the population who suffer from active yaws lesions within a period of 21 months is 52·2. The actual incidence of the disease, of course, is far higher than this, and could only be estimated accurately by separating the figures for the age group 2 to 12 years at which infection is most likely to be active. Satisfactory statistics for this computation are unfortunately not available.

It was hoped to estimate also the age of onset of the disease and the likelihood of a child contracting the infection in the first 2, 3 or 4 years of life. A follow-up was attempted of all babies born from June, 1935, to June, 1939. It was found that of 407 children born in this period, 253 could be traced for 2, 3 or 4 years, only 23 or about 9 per cent. were free from yaws infection, and most of these negative cases were traced for only two years. Unfortunately the presence of the large group of 154 births not traced prevents the drawing of final conclusions as to the average age of incidence, since in this group it is probable though not certain that a much larger percentage was free from infection. It can only be said that of all children living to the age of 4 years a proportion that is at least 60 per cent., and probably much closer to 90 per cent., is infected by the yaws organism. The writer has seen only two cases of an unquestionable primary lesion occurring in an adult.

STANDARD TREATMENT OF YAWS.

The figures for this and the following sections of this paper were obtained from the records of all patients, irrespective of dwelling place or of creed, diagnosed as suffering from active yaws, in whom sufficiently detailed records had been kept. The total number of patients so diagnosed was 828, but of these 290 were rejected on account of incompleteness of the records. A total of 538 fully recorded cases remains for analysis, and these were divided into three groups of cases as follows :

1. *Secondary Yaws.*—This term is taken to include all cases of frank secondary eruption, whatever the age of the patient; also condylomata and similar lesions in children under 2 years of age. This age limit was chosen arbitrarily because of the difficulty of drawing a hard and fast line between

late secondary and early tertiary lesions in patients not observed throughout the whole process of evolution of the disease. A few cases of primary yaws— a condition not often seen at the hospital as parents wait for the secondary eruption before bringing patients for treatment—is also included in this section.

2. *Early Tertiary Yaws.*—All cases, except those of frank secondary eruption, in children of from 2 to 12 years of age. These include single and multiple ulcers, condylomata and other muco-cutaneous lesions, desquamating plantar and palmar yaws, periostitis diffuse and nodular, and other less common lesions. No lesions differing greatly from those described in other parts of the world have been observed.

3. *Late Tertiary Lesions.*—All cases, except those of frank secondary eruption, in patients over 12 years of age.

The previous history of native patients is almost impossible to obtain with any accuracy. Hence in the sections dealing with the efficiency of injection therapy the following method has been used : each separate occasion of coming for treatment by a series of injections, whether treatment had been given previously or not, was taken as a test of the efficacy of the drug, provided that the interval since last receiving treatment was more than 1 month, during which time any effective depot in the body might be considered to have disappeared. The numbers in the tables below refer, therefore, to " Treatments," *i.e.*, to series of injections, and not to individual patients. Only in the section on relapses are individual patients referred to. Some patients in the period under review received as many as six separate treatments for successive relapses, and in this way the 538 cases have provided a total of 810 " treatments " for analysis.

1. SECONDARY YAWS.

This group, defined above, included ninety-nine cases giving a total of 115 " treatments " for analysis. They are divided into two classes, those receiving " T1 " (three or more consecutive injections), and those receiving " T2 " or " T3," the less satisfactory treatments as described above. The results are set out in Table I, which compares the two groups with respect to the immediate result and also the relapse rate over a period of 18 months' observation. The final column shows for convenience the number showing no relapse at the end of this period.

The numbers in this group are too small for the calculation of percentages ; but expressed in round figures it will be seen that with the standard treatment of three consecutive injections about three-quarters of the cases can be healed. (As noted later, the majority of cases not responding to three injections will be completely healed by a further one or two injections, provided that the weekly continuity of the series is not interrupted.) The immediate results of treatment are therefore good. The latter part of the table shows, however,

Mission, most of them being Seventh Day Adventists ; many of these receive treatment at the Methodist hospital, but in this calculation they have been omitted from both numerator and denominator, as the proportion receiving treatment at other centres is not known. Their numbers are not great enough, however, to affect the result to a significant extent, nor is there any reason to believe that the incidence of the disease varies significantly in the two groups of the population.

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Type D .. Bony yaws, frank periostitis nodular or diffuse, or in the case of adults typical bony pains, diagnosed as yaws on the grounds of previous history, signs of latent yaws, and the response to treatment.

In this age group, 3 to 12 years, there were only five cases of Type C lesion ; these have therefore been added to the Type C cases in the following section and omitted here. Type D cases numbered only twenty-one and have been considered together with Type A. The number of patients in this section was 244, providing a total of 408 " treatments " for analysis. The distribution of cases and the results of treatment are set out in Table. II for " T1 " treatment, and in Table III for " T2" and " T3." The conventions are the same as in Table I.

TABLE II.

Effects of " T1 " in Early Tertiary Yaws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
A D } 203	135	68	80	28	40	51	63	74	6
B 104	85	19	46	9	21	27	31	35	11
Total 307	220	87	126	37	61	78	94	109	17

It will be seen from this table that of 203 Type A (including fourteen Type D) cases, 135 or 67 per cent. were relieved ; and of 104 Type B cases, 85 or 81·8 per cent. were relieved by three consecutive injections. In relapse, however, there is little difference between the two types of lesion, both types showing about half the cases relapsing within 6 months. The proportion of cases still free of relapse after 18 months is considerably higher in Type B, but the small number of cases renders this difference of doubtful significance. The findings again confirm the clinical impression that ulcerative yaws in children is less susceptible to treatment than the common plantar lesions, and that relapse is distressingly frequent in both types after the standard treatment of three consecutive injections.

In Table III are shown the comparable figures for the much smaller numbers receiving " T2 " and " T3 " types of treatment.

The inferiority of the results from " T2 " and " T3 " can be readily seen from this table, but to facilitate comparison between the two types of

TABLE I.

Effects of Sobita in Secondary Yaws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
T1 88	66	22	41	14	20	27	32	35	6
T2 T3 } 27	16	11	12	7	9	12	12	12	0

Tables I to X.—Numbers in the first column represent the total number of cases in each class. R = Relieved.   NR = Not relieved.

that of the cases completely healed—only completely healed cases were considered in the enumeration of relapses—approximately half will have relapsed, either with further secondary eruption or with early tertiary lesions, within 6 months of completing treatment, while very few will be free of relapse at the end of the test period of 18 months.   When the less satisfactory courses of treatment are given the immediate " cure " rate is practically as high.   No cases followed up, however, were free from relapse at the end of 9 months.

This finding fully confirms one's clinical impression that the secondary eruption is extremely susceptible to sobita, but that relapse is distressingly common.   It is, however, distinctly less common when the standard treatment of three consecutive injections is given than when less complete treatment is attained.

2. EARLY TERTIARY YAWS.

The cases included in this group have already been defined ; treatment throughout was with sobita in the proportionate dosage described above.   In this and in the following section the cases have been divided primarily into four groups according to the type of lesion present.   These four groups, designated A, B, C and D, were studied separately to discover whether any significant difference existed between the susceptibility of different types of lesion to injected drugs.   The four groups are constituted as follows :

Type A   .. Ulcerative yaws on limbs or trunk.

,,   B   .. Plantar yaws, comprising two main lesions ;   the relatively superficial lesion marked by desquamation cracks and fissures, known to the natives as " hotihoti " ;   and the classical " crab yaws," called " bolivu " in the Roviana language.

,,   C   .. An uncommon desquamating lesion on legs or arms, marked by its superficial nature and serpiginous outline.

Type D .. Bony yaws, frank periostitis nodular or diffuse, or in the case of adults typical bony pains, diagnosed as yaws on the grounds of previous history, signs of latent yaws, and the response to treatment.

In this age group, 3 to 12 years, there were only five cases of Type C lesion ; these have therefore been added to the Type C cases in the following section and omitted here. Type D cases numbered only twenty-one and have been considered together with Type A. The number of patients in this section was 244, providing a total of 408 " treatments " for analysis. The distribution of cases and the results of treatment are set out in Table II for " T1 " treatment, and in Table III for " T2" and " T3." The conventions are the same as in Table I.

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In Table III are shown the comparable figures for the much smaller numbers receiving " T2 " and " T3 " types of treatment.

The inferiority of the results from " T2 " and " T3 " can be readily seen from this table, but to facilitate comparison between the two types of

<div align="center">TABLE III.</div>

<div align="center">Effects of " T2 " and " T3 " in Early Tertiary Yaws.</div>

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
A D } 77	39	38	38	9	21	28	33	34	4
B 25	12	13	11	4	6	8	11	11	0
Total 102	51	51	49	13	27	36	44	45	4

treatment, a comparison more important than that of the differing susceptibilities of the different types of lesion, the totals from each table have been summarized and placed in apposition in Table IV.

<div align="center">TABLE IV.</div>

<div align="center">Comparison of " T1 " with " T2 " and " T3 " in Early Tertiary Yaws.</div>

Treatment.	Number Relieved.	Traced.	Number Relapsed in		No Relapse.
			6 months.	18 months.	
T1 307	220 (71·7 per cent.)	126	61	109	17
T2 T3 } 102	51 (50 per cent.)	49	27	45	4

It will be seen from this table that whereas in " immediate result " the " T1 " treatment gives the fairly satisfactory figure of 71·7 per cent. relieved as against only 50 per cent. with the less complete types of treatment, yet when the relapse rate is considered there is little difference between the two treatments, both being equally unsatisfactory, since in each case half the patients· have relapsed within 6 months and nine-tenths within 18 months.

3. LATE TERTIARY YAWS.

The number of cases in this group was 210, and the total number of treatments for analysis 287. The lesions have been classified into four groups as defined in the previous section and the cases again divided into two sections according to the drug used in treatment. The conventions T1, T2 and T3 are used in the same sense as previously, both for sobita and for N.A.B.

A. *Treatment with Sobita.*

The results of sobita treatment in this group are set out in Tables V, VI and VII, which are directly comparable with Tables II, III and IV respectively, save that separate figures have been given for each of the four types of lesion.

TABLE V.

Effect of " T1 " (Sobita) in Late Tertiary Yaws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
A 39	32	7	12	1	4	4	7	7	5
B 47	33	14	15	1	3	7	8	9	6
C 4	4	0	1	1	1	1	1	1	0
D 22	19	3	6	3	3	3	3	5	1
Total 112	88	24	34	6	10	14	18	22	12

TABLE VI.

Effect of " T2 " and " T3 " (Sobita) in Late Tertiary Yaws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
A 10	7	3	4	0	2	3	3	3	1
B 16	11	5	5	1	4	5	5	5	0
C 4	2	2	0	—	—	—	—	—	—
D 15	10	5	6	2	5	6	6	6	0
Total 45	30	15	15	3	11	14	14	14	1

TABLE VII.

Comparison of " T1 " (Sobita) with " T2 " and " T3 " (Sobita) in Late Tertiary Yaws.

Treatment.	Number Relieved.	Traced.	Number of Relapsed in		No Relapse.
			6 months.	18 months.	
T1 112	88 (78·6 per cent.)	34	10	22	12
T2 T3 } 45	30	15	11	14	1

The numbers are small, but some conclusions may be drawn. As with the earlier tertiary lesions, the standard three injection treatment gives an "immediate cure" rate of 78·6 per cent.; about a third of the cases traced showed no relapse after 18 months, which is a distinct improvement on the relapse rate in the earlier age group. Less efficient treatment gives an "immediate cure" rate only slightly lower, but only one of fifteen cases traced was free of relapse at the end of the 18 months. It is evident from these figures that the tendency to relapse in late tertiary yaws is less marked than in the earlier lesions, but that three injections is the minimum at which treatment can be considered in any way effective.

B. *Treatment with N.A.B.*

Tables VIII, IX and X set out in parallel fashion the results in the smaller group of cases treated with N.A.B.

TABLE VIII.

Effect of " T1 " (N.A.B.) in Late Tertiary Yaws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR		3 months.	6 months.	9 months.	12 months.	18 months.	
A 22	17	5	10	2	2	2	5	5	5
B 23	19	4	15	2	6	6	7	8	7
C 4	4	0	1	1	1	1	1	1	1
D 28	25	3	14	1	1	6	9	9	5
Total 77	65	12	40	5	9	14	21	22	18

TABLE IX.

Effect of " T2 " and " T3 " (N.A.B.) in Late Tertiary Laws.

Treatment.	Immediate Result.		Traced.	Number of Relapses occurring within					No Relapse.
	R	NR	months.	3 months.	6 months.	9 months.	12 months.	18 months.	
A 4	4	0	6	0	3	4	5	6	0
B 14	13	1	10	1	3	5	7	7	3
C 4	4	0	1	0	1	1	1	1	0
D 16	14	2	11	0	5	7	9	11	1
Total 38	35	3	28	1	12	17	22	25	3

TABLE X.

Comparison of " T1 " (N.A.B.) with " T2 " and " T3 " (N.A.B.) in Late Tertiary Yaws.

Treatment.	Number Relieved.	Traced.	Number of Relapses in		No Relapse.
			6 months.	18 months.	
T1 77	65	40	9	22	18
T2 } 38 T3	35	28	12	25	3

The results are seen to be closely comparable with those of sobita treatment. The three injection treatment gave an " immediate cure " rate of 65 out of 77, and of 40 cases traced there were 18 without relapse in 18 months. With the less complete treatment the " immediate cure " rate is higher than in any other series examined, but the relapse rate after 18 months is the same as in the other two groups.

Comparison of the Two Drugs.

Careful comparison of these two series of tables brings out the following points : For long-term results a minimum of three injections is just as necessary with N.A.B. as it is with sobita. When only one or two injections can be given, N.A.B. is admittedly more effective in producing immediate healing of the lesion, but the relapse rate is as unsatisfactory as with sobita used in the same way. When the standard three-injection treatment is used there is little difference between the two drugs either in the " immediate cure " rate or in the occurrence of relapses within an 18-month period of observation. The figures for relapses are too small for accuracy but do suggest that relapse may be more delayed after N.A.B. than after sobita.

Susceptibility of Different Lesions.

The tables show no significant difference between Type A and Type B lesions in their reaction to either drug, but bring out well the very ready response of Type D—those with generalised bony pains, whether with or without frank periostitis. Special care was taken to exclude cases with a doubtful diagnosis, and those in which a psychologically suggested subjective improvement only might have occurred. Even so, the " immediate cure " rate was 18 out of 22 for sobita and 25 out of 28 with N.A.B., and when only one or two injections were given the rate was still as high—11 of 15 with sobita and 14 of 16 with N.A.B. The relapse rate, however, was similar to that of the other types of lesion.

Type C was present in such small numbers as to be valueless for comparison, but it was examined separately because it is an interesting lesion, not

fitting into the other categories, and one had the clinical impression that it was extremely responsive to treatment, often disappearing completely after one or two injections. The small numbers available confirm this impression.

<p style="text-align:center">EFFECT OF FOUR, FIVE AND SIX INJECTIONS.</p>

Two questions were investigated under this heading, with the object of exploring possible modifications of the standard treatment: (a) Whether a lesion which has failed to respond to three consecutive injections is likely to benefit by a further continuation of treatment; and (b) whether the prolongation of treatment has any significant effect on the relapse rate. Figures for the three stages of the disease have been considered together in this section, and all cases are those which had had three injections without significant improvement.

(a) Effect of Continued Treatment on Refractory Lesions.

Seventy-three cases were available for study, comprising 11 secondary yaws, 55 early tertiary lesions, and 7 late tertiary. The smallness of the number —only 73 out of a total of 235 in the tables classed as " not relieved "—is an indication of the difficulty of getting the natives to stay for more prolonged treatment, even though their lesions are still active. The history of these cases is set out below in a form which expresses the results rather more clearly than a formal table :—

> 73 cases received 4 injections : 16 were relieved, 2 not traced.
> 55 cases received 5 injections : 16 were relieved, 12 not traced.
> 27 cases received 6 injections : 16 were relieved, 4 not traced.
> 7 cases received 7 injections : 5 were relieved, 1 not traced.
> 1 case received 8 injections, and was relieved by the eighth.

Thus excluding the nineteen cases who were not traced, of fifty-four patients attending for longer periods up to a maximum of 8 weeks, all were healed. And one's experience enables one to say that in very few of these cases would spontaneous healing have occurred in such a short time ; thus a number of those " not traced " were seen at intervals of months later, with their lesions practically unchanged. The figures, though small, provide strong evidence for continuing injections for at least 8 weeks in refractory cases.

(b) Relapse Rate After Longer Treatment.

This is actually the most important question to be answered : since if good evidence were forthcoming that a longer course of treatment could effect a substantial reduction in the relapse rate, it would be worth while attempting to persuade those who had already healed with three injections to continue treatment in the hope of avoiding relapse. The number of cases treated and

traced was unfortunately small on account of the difficulties already referred to : a period of 18 months was considered the minimum for assessing relapse, since the inconvenience of having to stay for a 6 to 8 weeks' course of treatment would not be adequately rewarded by merely delaying relapse for a few months. The natives would prefer to come for two periods of three weeks at intervals of a few months rather than to stay the longer period at one time. The results are set out in Table XI.

TABLE XI.

Relapse Rate after Prolonged Treatment.

Number of Injections.	Number of Cases.	Relapse in 9 months.	Relapse in 18 months.
4	11	6	11
5	9	6	7
6	10	6	9
7 and 8	2	2	2

There is thus no evidence from these figures that the relapse rate is less with the prolonged period of treatment. It should be noted, however, that these were all refractory cases, though all ultimatedly healed by their prolonged treatment. A more accurate appraisal could only be made by giving the prolonged treatment to cases already healed by the standard three injections. It is now our practice to give at least four injections to all cases whenever possible : the period of follow-up is not yet long enough for an assessment of the results of this treatment.

SUMMARY AND CONCLUSIONS.

Over a period of 2 years at least half of the population of the islands of Vella Lavella and Ranono suffer from active yaws lesions, either cutaneous or bony. Extensive observations throughout the other islands of the Western Solomons suggest that a similar condition obtains throughout that part of the group.

A standard treatment of three consecutive injections of sobita (sodium bismuthyl tartrate), will produce healing in 66 per cent. of secondary lesions, in 71 per cent. of early tertiary, and in 78 per cent. of late tertiary manifestations. Most cases not healed by three injections will respond to treatment continued for longer periods up to 8 weeks. Less than three injections, or injections given at longer intervals than a week, are much less effective, though even by such means about 50 to 55 per cent. of " immediate cures " can be effected. N.A.B. used in adults gives very similar results when three injections are given (84 per cent. " immediate cure "), and when only one or two injections can be given in series is definitely more effective than sobita.

The relapse rate after the standard three injection treatment is high within 6 months about half of the cases have relapsed, and in 18 months, more than 17 per cent. of cases were free from relapse. The tendency to relap is considerably less in the late tertiary than in the earlier stages of the di. There is no significant difference between N.A.B. and sobita as used in u investigation in the time of occurrence or the frequency of relapses.

There is some difference in the susceptibility of the various types of lesi to the treatment. In both early and late stages the plantar yaw, which is most disabling and crippling condition, responds more readily than do u' ι. lesions in other parts of the body, and in the late tertiary stage of the dis the bony pains so often complained of are relieved more frequently th any other type of lesion. The rather uncommon serpiginous desquamati lesions of legs and arms are also extremely sensitive to treatment whether N.A.B. or sobita.

In cases not responding to the standard treatment of three injections, pe sistence of treatment will effect healing in the great majority of cases. The is, however, no evidence that the relapse rate in cases so treated is any mo satisfactory than in those receiving only three injections.

In consideration of the enormous difference in the cost of the two d there seems to be no justification for the routine use of N.A.B. in adult ca It should be used in those cases where the patient cannot possibly stay for full course of treatment, and possibly for a few cases, refractory to one dru which might respond to a combination of the two.